Studies on Holkham salts hole : an isolated salt water community with relict features

Thesis

STUDIES ON HOLKHAM SALTS HOLE:

AN ISOLATED SALT-WATER COMMUNITY

WITH RELICT FEATURES.

Barry R.Alcock,B.Sc.(Sp.Hons.),London.

Ph.D. Thesis - The Open University

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SUMMARY

There is at Holkham, North Norfolk, a marine pond which supports a fauna thought to be principally a relict from the time when it was part of an estuarine tideway, two hundred and fifty years ago.

Studies have been carried out on the hydrology and hydrography of this pond, known locally as the Salts Hole. The nature of the substratum, changes in salinity, temperature and other physical variables have been assessed and from these it has been possible to establish that conditions within the pond are remarkably stable.

The fauna is reviewed and its relationship to other brackish water ponds discussed.

Experiments designed to produce response surfaces have been carried out for three crustacean species, Idotea chelipes, Gammarus duebeni and Praunus flexuosus. These describe how changes in salinity, temperature and oxygen concentration influence survival of both adults and juveniles. Populations drawn from the surrounding marshes of Holkham Bay have been compared to those of the pond. The response surface centres of the Salts Hole populations correlate more closely with the conditions prevailing in the pond, than do the Bay populations.

Electrophoresis of Malate dehydrogenase and Leucine aminopeptidase isozymes was performed for the same three species of crustaceans. The Salts Hole populations contain fewer alleles and show significantly higher levels of genetic divergence. Several hypotheses relating genetic polymorphism and environmental conditions have been examined. The findings support Valentine's hypothesis of trophic resource stability and Stenseth's interpretation of the Red Queen hypothesis, predicting that fewer species are found in the pond than in the surrounding salt-marshes.

The relative contributions of random genetic drift and selection are discussed and conclusions are drawn that the Salts Hole populations, despite their relatively short period of isolation and small numbers, have been subjected to the effects of selection.

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TABLE OF CONTENTS

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LIST OF ILLUSTRATIONS. (a) Diagrams.

(b) Plates.

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Plate 1. View of the Salts Hole from the dunes: The culvert is visible in the background.

INTRODUCTION

Holkham Salts Hole, near Wells, Norfolk, (map reference TF 886451), is a salt-water pond which has been isolated from the sea, except in conditions of extreme flooding, for about 250 years. It supports a fauna similar to those recorded from other brackish pools which have become permanently or temporarily isolated from the sea or estuaries. Despite the considerable body of work which has been carried out on the biology of estuarine animals, little has been published on these isolated communities. Gurney (1923) briefly described a brackish-water pond at Salthouse, Norfolk, which contained the sea-anemone Sagartia luciae. Verril, and Ellis (1932) commented on the fauna of Widewater, a lagoon cut off from the Adur estuary at Shoreham, Sussex. More extensive studies were carried out on the fauna of New England Creek, Essex, (Howes, 1938), and on that of a saline lagoon not far from the Salts Hole at Titchwell, Norfolk, (Williams, 1972).

The Salts Hole was first referred to in a paper (Pantin 1965), in which the pond was used as an example of steady state maintenance in the physical environment. Pantin had studied the Salts Hole between 1963 and 1966 as a spare-time and holiday project and he investigated its nature, origin and ecology. He died in 1967 before he could write up his work, but from his notes and illustrations, Hunt (1971) compiled an account of the pond.

The Salts Hole lies amongst marshy fields immediately adjacent to, and on the landward side of the sand-dunes known as Holkham Meals on the Earl of Leicester's estate, (plate 1). The dunes have existed in their present position for several centuries (Steers 1934). They are well consolidated, having been planted with conifers during the last century. Throughout the period of exact mapping, the shore has prograded, and sand has accumulated on the seaward side.

The pond is broadly ovate in shape, approximately 100 m in length and 70 m broad. The maximum recorded depth is 2.7 m. It is fed by a salt-water spring entering its eastern shore and issuing from the base of the sand-dunes. Water leaves the pond over a dam situated at the south-western end and drains by way of a ditch through the marshes.

Close to the northern boundary is a much smaller pond with which the Salts Hole communicates. This has become largely infilled. The sea rush Juncus maritimus Lam. now covers the whole of its surface and its maximum depth is less than 15cm.

SALTS HOLE: plan

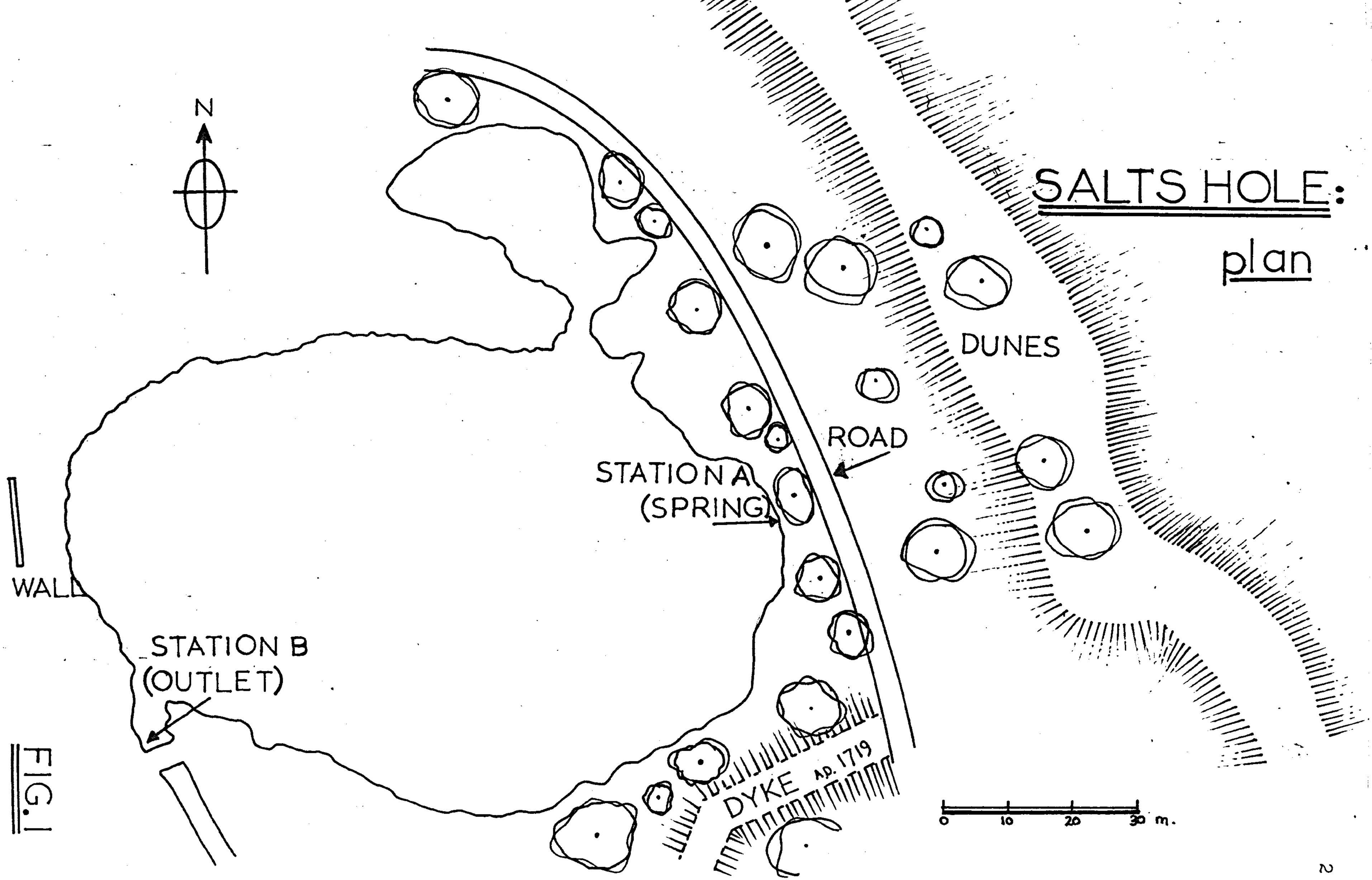


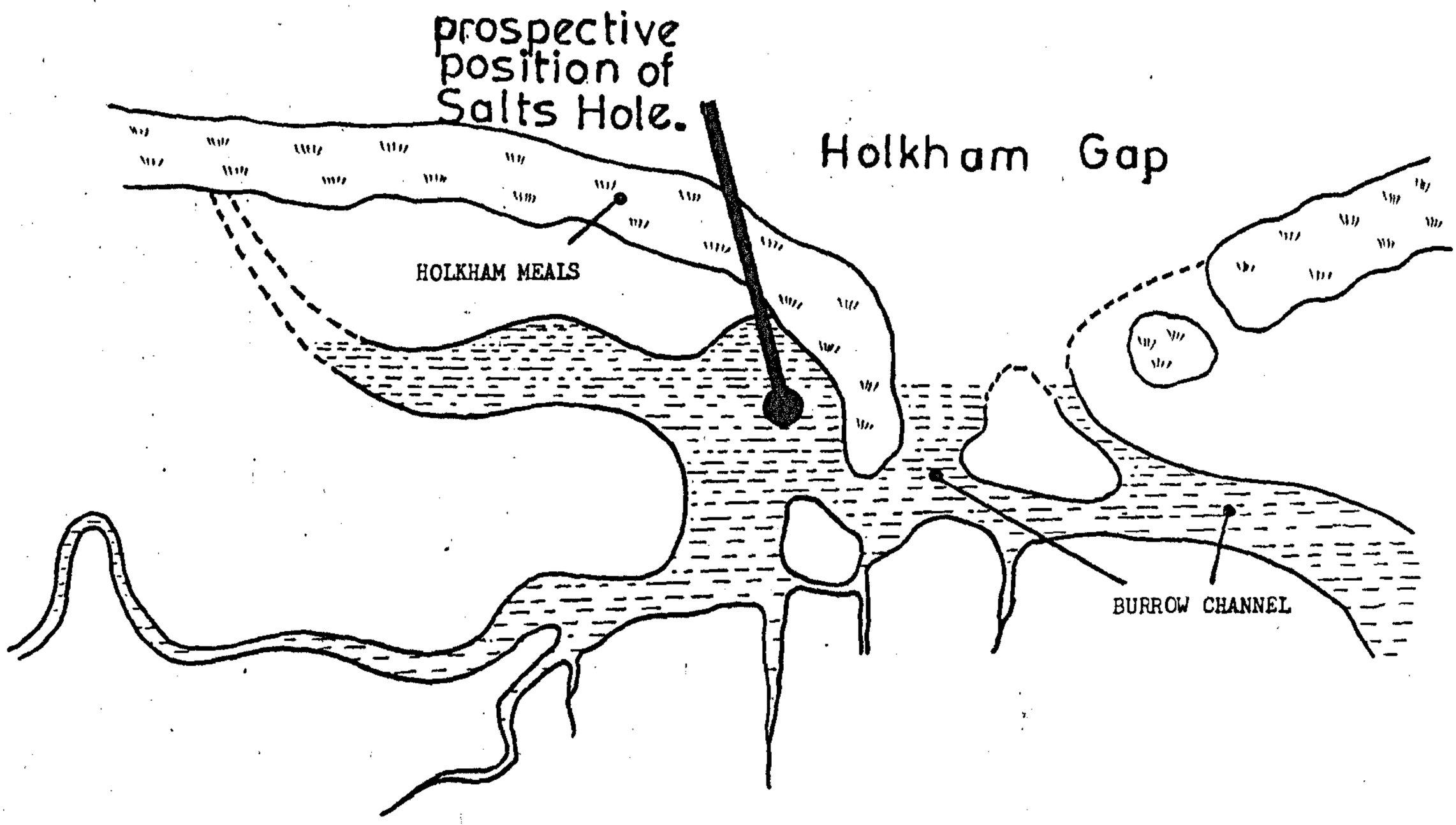
FIG. 1

Steers (1934, 1951 and 1960) in describing the nature and development of the coastline of north Norfolk has shown how mobile shingle and shell ridges are built up from the off-shore sand flats by wind and wave action. Consolidation by vegetation leads to further accretion of sand until fringing sand-dunes are formed. An important feature of this coastal development is the way in which the shingle barriers grow and change shape; their extremities curving landwards to form terminal spurs and lateral ridges which project into the associated saltmarshes. Examples of this process may be seen at Blakeney Point, and at Scolt Head island. Frequently a portion of a marsh or a creek system is cut off by the incurving spur of shingle or sand-dune. The river Glaven, for example, at Cley-next-the-sea, has been progressively diverted in this manner since mediaeval times. The Salts Hole was undoubtedly once part of the creek system that existed as a tideway into the saltmarshes before these were enclosed by artificial embankments in 1719 to become the grazing fields they are today. The position of the old creek system may still be traced from aerial photographs. Even before 1719 the entrance to the tideway was being progressively narrowed by sand-dune spurs closing the channel (figure 1a) and with the construction of the embankment, the Salt-Hole was in effect isolated from the sea. (figure 1b) The Salts Hole is first recorded on a map of Wells Harbour dated 1780, but it seems most likely that its origins go back to the time when the saltmarshes were drained in 1719. Possibly it was enlarged then to act as a containing reservoir for the saline water permeating through the dunes, which, if left to escape, would have been detrimental to agriculture. The Salts Hole still carries out this function today. The excess water passing over the dam into the drainage ditch finally reaches the sea at Wells. The dam and culvert system were constructed in 1834. At the same time the pond acquired its present ovoid shape, losing a southward projecting arm which is recorded on the earlier maps.

OBJECTIVES

Most of the key studies examining the genetical basis of evolutionary changes have been derived from the investigations of a relatively few terrestrial species, representing a fraction of protist, plant and animal taxa. There has never been any reason to postulate a difference in the genetic systems of marine and terrestrial organisms, but the apparent uniformity of the open sea, with its less prominent barriers to movement and dampened environmental fluctuation, has provided the basis for speculation that evolution and spec-

(a)



DEVELOPMENT OF THE SALTS HOLE

(A) SHOWING SITE OF SALTS HOLE PRIOR TO THE CONSTRUCTION OF DYKES IN AD 1690.

(B) THE BUILDING OF THE DYKES FINALLY ENCLOSED THE POND IN AD 1719.

(b)

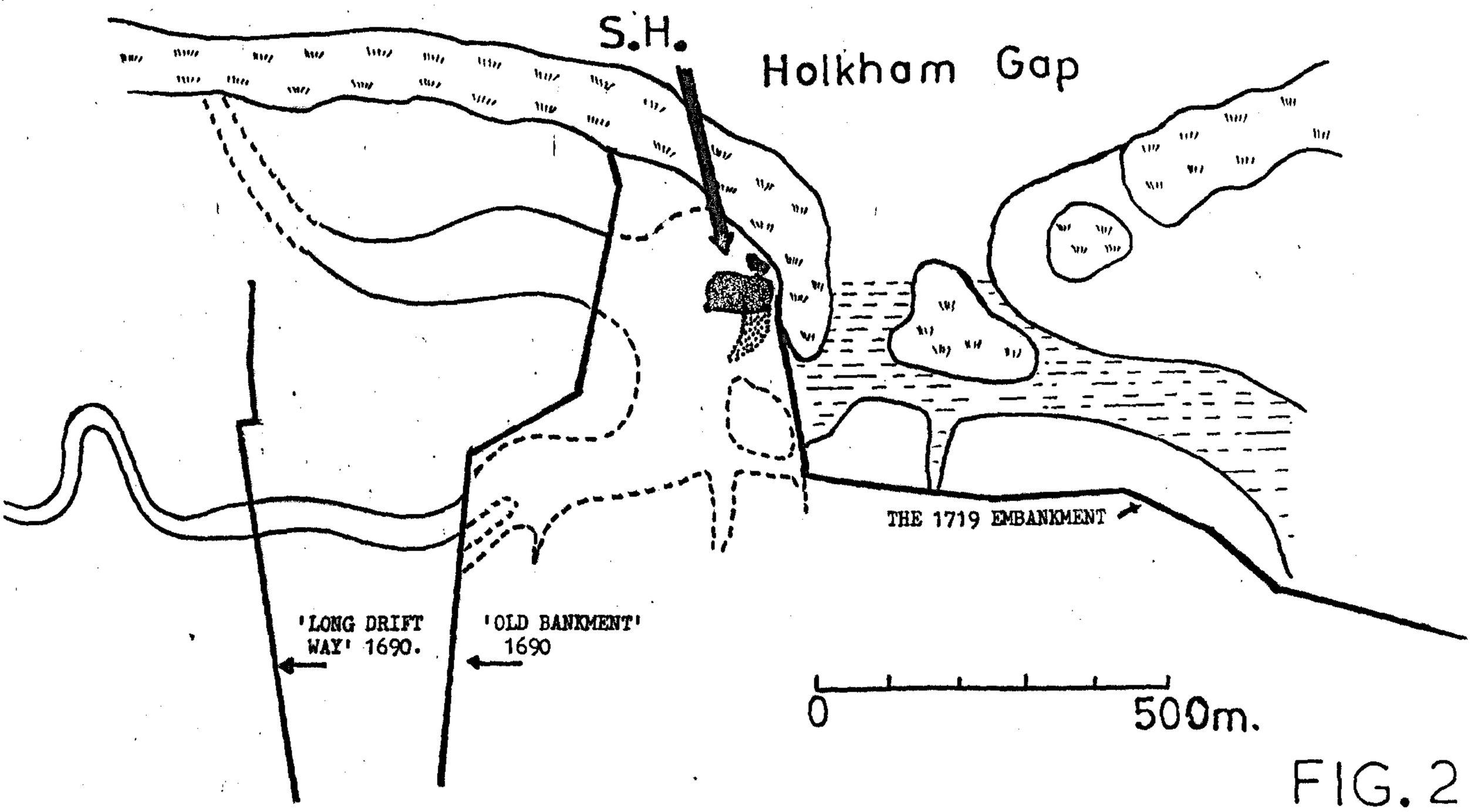


FIG. 2

iation might be subtly different there. Zeuner (1958) argued from fossil evidence that the tempo of evolution is much slower in the sea than on land. Sympatric speciation, or speciation by distance over a continuously occupied range, has been proposed for marine organisms (Kohn 1960; Day 1963).

It is increasingly evident that barriers to free movement are widespread in the sea. These include temperature and salinity differences of water masses, low nutrient regions, inimical substrata, countervening currents and the dwarfing of dispersal efforts by oceanic distances. The degree of permanence of these barriers in respect to time is difficult to assess however, and this has made the task of the marine geneticist very difficult. In the editorial introduction of Speciation in the Sea, (Harding and Tebble,1963) it is stated:

> 'The nature of the sea as an environment imposes experimental limitations which never arise in terrestrial habitats and which are difficult to overcome. The almost complete lack of genetic information and the very great difficulties in the way of obtaining it, makes the distinction between phenotypic and genotypic differences very difficult to assess.'

In the years since that statement was published, much more has been discovered about the physiological variation of geographically separated populations, but the genetic components of this variation have proved difficult to disentangle from physiological adaptation.

The Salts Hole, cut off from the sea and presumably protected from vigorous environmental fluctuation, presents a fortuitous opportunity to study the influence of genetic and physiological adaptive mechanisms in maintaining an isolatedfauna. The concept of the intrinsic adaptiveness of high genetic variability is often cited by evolutionists, usually without recourse to causal explanations of the origin of variability. The core of the arguement is that genetic variability is a form of insurance against environmental perturbations. Among the multitude of genotypes arising each generation from recombination and syngamy exist combinations potentially adaptive over a wider range of environmental conditions than the population actually experiences. Under a new environmental stress the appropriate alleles are mobilised, enabling a fraction, at least, of the population to survive and propagate. The 'price' for this insurance is exacted each generation as a segregational load of non-adapted genotypes. This genetic load may, in fact, be spurious because low-fitness genotypes may comprise most of the inevitable ecological

deaths, the 'ecological load' of each generation. (Turner and Williamson 1968: Wallace 1970). This phenomenon of genetic variability is linked together with species diversity and environmental predictability in hypotheses of genetic-adaptive strategies.

(Sanders 1968) distinguishes physically controlled communities typified by high diversity. In the former, environmental variation is often erratic and severe. A premium is placed on adaptation to fluctuating physical stress and adaptation to the biotic community is secondary. Species that successfully adapt are relatively few and each is able to appropriate a large share of resources and to maintain large numbers. Shallow continental shelf and estuarine communities are of this type, even in regions of high productivity. Biologically accommodated communities occupy uniform or highly predictable habitats, in which time and stability permit the evolution of complex and subtle species interaction. The first part of this study sets out to show that the Salts Hole undoubtedly has the stability associated with biologically accommodated communities. Has its mere 250 years of existence had time to leave any mark on the fauna in terms of genetic variability? This will be examined by studies relating to fitness in response to the effects of environmental variables acting in concert, in selected species of its fauna. Further studies examine the degree of genetic polymorphism (genetic variability, sensu stricta), in these same species.

(Grassle 1972) bases adaptive strategies of marine species on the amount of genetic variability. Species of more physically controlled communities possess high genetic variability. This is presumably generated by environmental heterogeneity and maintenance is made easier by the large population size of the relatively few species. Genetic variability is viewed in its insurance aspect; some genotypes - temporary and fortuitous elites - survive each unpredictable stress and perpetuate the species. The estuarine fauna of Holkham Bay must represent such a community. A similar but more far reaching hypothesis of genetic-adaptive strategies is posited by Bretsky and Lorenz (1970). They assume that environmental stability in time promotes selection for homozygosity or homoselection. (Carson, 1959, uses this in a different sense). Conversely environmental instability generates genetic variability through heteroselection. Environments such as estuaries are primarily heterogeneous and unpredictable and species in these communities contain high genetic variability, again as insur-

ance for species survival.

Prolonged homoselection in stable high-diversity communities leads to fine adjustments in species relationships, but also increases vulnerability to extinction in periods of environmental vicissitude. There is evidence that diverse faunas of stable habitats in the fossil record have had less geological persistence than low-diversity heteroselected communities, which, to some extent, were preadapted to environmental change (Bretsky 1969). These events take place on time-scales measured in millions of years. Is it realistic to expect the Salts Hole fauna to show any trend towards homoselection in 250 years?

There is also the factor of population size to take into account. The Salts Hole community is very small. (Ohta 1974) points out that small selection coefficients will be ineffectual in small populations, rendering the corresponding genotypes effectively neutral and unable to silence random genetic drift. This will obviously lead to difficulties in interpreting the results from the Salts Hole. It nevertheless emphasizes the potential value which studies on its fauna could have in throwing light on the relative merits of several hypotheses on genetic diversity, and this is the prime object of this thesis.

The experimental work divides into two major categories. In the first, a survey of the conditions prevailing in the Salts Hole and the nature of its fauna is undertaken. This is to establish whether it really is the biologically accommodated community it purports to be. The second part examines the degree of genetic variability in three common Salts Hole species by studying their survival response to physiological stresses and by calculating their indices of genetic diversity with respect to two enzyme polymorphisms. Comparative studies are carried out on animals of the same species drawn from the physically controlled communities of Holkham Bay.

HYDROGRAPHIC STUDIES

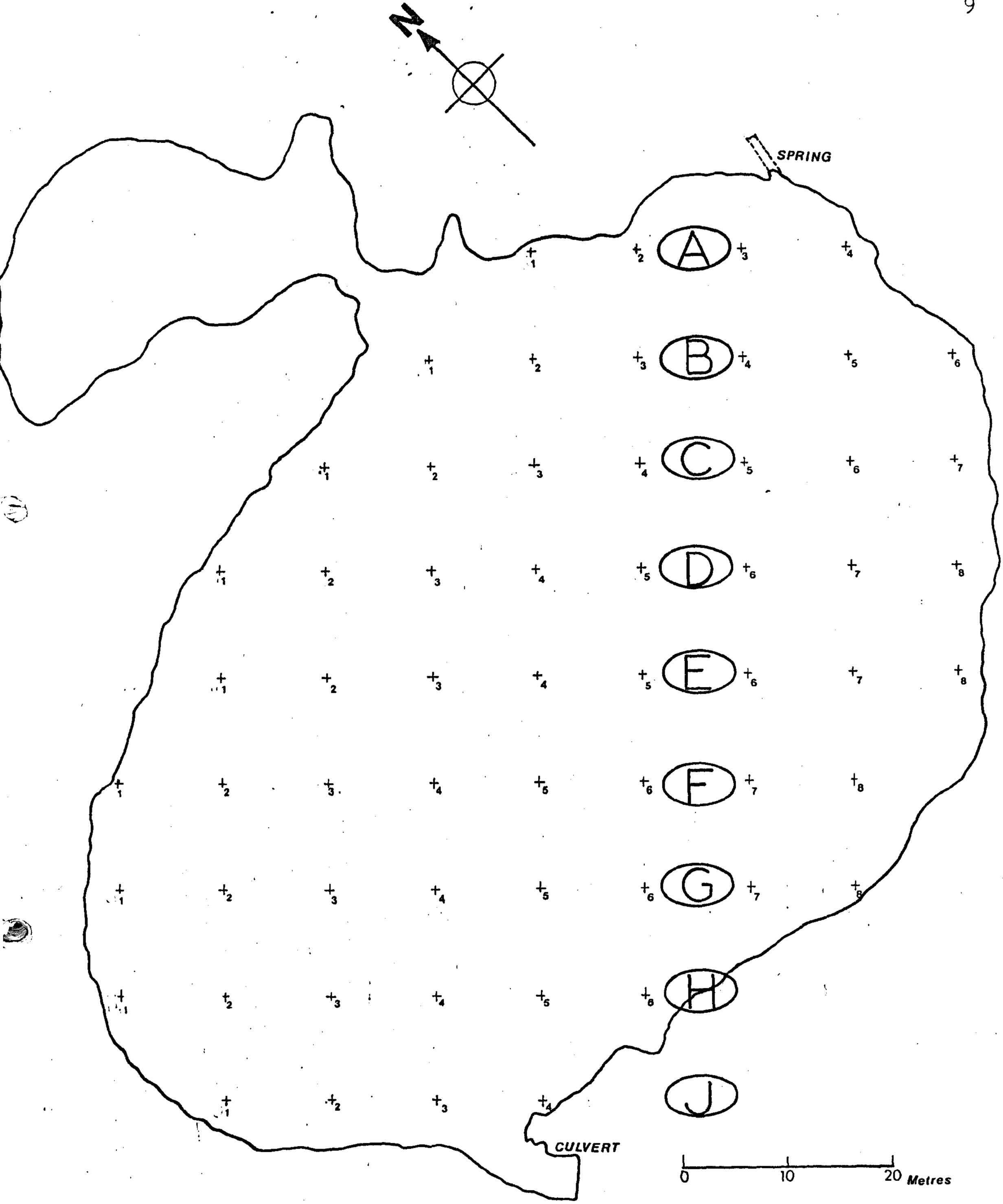
Certain environmental variables, namely the salinity, temperature and hydrogen ion concentration of the water and its oxygen content, were monitored at approximately monthly intervals from April 1974 to April 1977. In addition a more comprehensive survey was carried out in March 1975 and again in October 1975 where a 10x10 m grid was marked out and recordings of the same environmental variables taken at 0.5m depth intervals, at the intersections.

Methods

The monthly samples were collected at the surface of the pond where the main spring enters (station A) and where the water leaves the pond (station B). These sampling stations are indicated in fig. 1. The salinity of the samples were analysed in the field using a T/C refractometer (A.O. Instrument Co., U.S.A.) calibrated for direct salinity measurement in parts per thousand NaCl. Oxygen content was monitored using a dissolved-oxygen meter (Type 15A, Electronic Instruments Ltd., G.B.) and recorded as mg oxygen per litre water. Hydrogen ion concentration was determined using a pH meter (Type EH 331, Irwin Ltd., G.B.) and temperature with a thermistor thermometer (Model K , Edale Instruments, G.B.)

In the hydrographic survey of March and October 1975, water samples were collected from the pond by boat at the intersections of a 10x10 m grid marked out by ropes stretched out across the pond. There were 59 stations from which samples could be taken (fig.3) and at each station recordings were monitored at 0.5 m depth intervals using a series of probes arranged in a frame for recording oxygen content, pH, and temperature. In order to measure the salinity, a wide bore capillary tube was lowered to the appropriate depth and by releasing pressure gently with an adjustable screw, the few drops of water necessary for a refractometer reading were withdrawn. The instruments used for monitoring the environmental variables were those previously described. The depth of the pond was also recorded at each station.

At each station a sample of substratum was collected using a Birge-Ekman grab (Mark II, Lockwood and Sons, G.B.) enclosing a square core of $0.1m^2$ surface area. The depth to which this grab penetrated varied with the nature of the substratum, but on average removed the top 15cm of material. The sample was divided into two subsamples which were immediately treated with 5% formalin in seawater. Both subsamples were sorted in the laboratory and all the macrofauna was removed and ident-



SALTS HOLE FIG. 3

sampling stations; (10m.grid)

ified. The subsamples were then dried to constant weight. Rapid particle analysis was then performed using the method described by Buchanan and Kain (1971) except that large pebbles and shells were first removed by retaining them on a 40mm mesh sieve. The silt portion passing through the final sieve of the series (62μm) was not further subdivided in most samples, but where this fraction exceeded 10% of the total, as it did at 5 stations, sedimentation analysis was carried out. The methodology of both these processes and an example of each are given in Appendix 1. After the sieving analysis had been completed, the values for each particle size from both subsamples were averaged, and a percentage cumulative frequency curve plotted for every station.

Particle size was plotted on the phi scale, a logarithmic scale derived by Krumbein and Pettijohn (1938). Examples of these frequency curves and the formulae for deriving substratum attributes from them are included in Appendix 1. The latter include a measure tendency, the degree of scatter and the degree of asymmetry of particle size distribution.

OBSERVATIONS

The Salts Hole is fed by a spring issuing from the south-west face of the dunes. The intertidal sands act as a sponge-like reservoir in which the water table is considerably above mean sea level and water filters through the dunes and into the pond. The main spring discharges at an average rate of 12 litres per min., varying from 9 l min^{-1} at neap tides to 12.5 l min^{-1} at the springs. Subsidiary springs must account for an approximately equal volume of water since the mean outflow of the pond is about 25 l $min.^{-1}$

Water entering the pond showed a remarkably constant salinity throughout the survey period. The highest recorded salinity was 28.4‰ and the lowest was 21.6‰. Similarly at station B, near to the outlet there was little variation in the samples with a maximum value of 26.3‰ and a minimum of 18.4‰. The results of the monthly salinity readings are shown in fig 4 and the full data is to be found in Appendix 2. Also included on this graph are the monthly rainfall figures from the nearest meteorological station at Cromer, Norfolk, (D6NN station No. 3069).

Oxygen content and hydrogen ion concentration monitored at the same stations as salinity are recorded in fig 5. The incoming spring water is virtually anaerobic and remains at a constant level of 0.82± 0.08 mg l^{-1} O_2 .

SALTS HOLE: MONTHLY SALINITY RECORDS
CROMER: MEAN MONTHLY RAINFALL

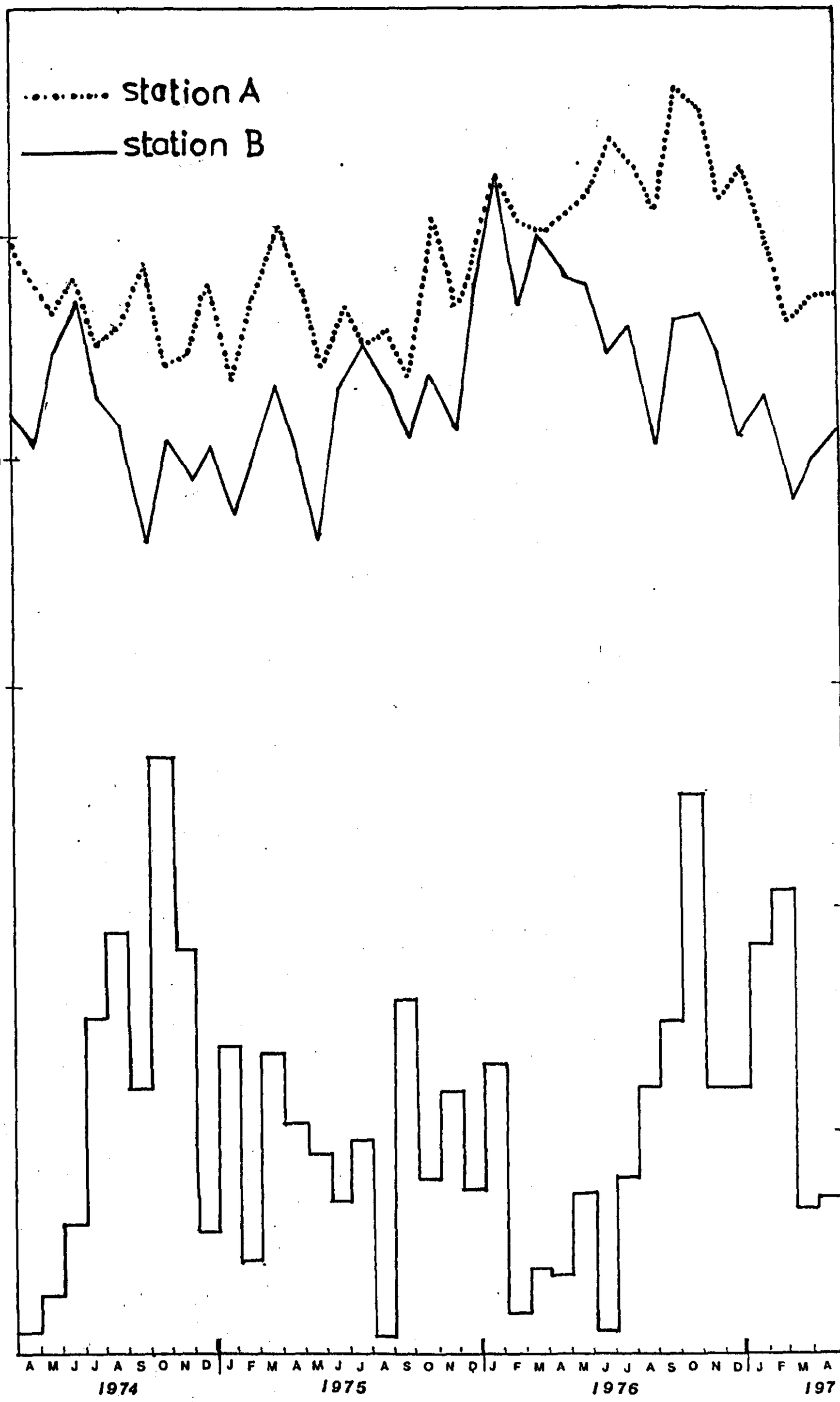


FIG.4

SALTS HOLE: MONTHLY pH AND OXYGEN CONCENTRATIONS.

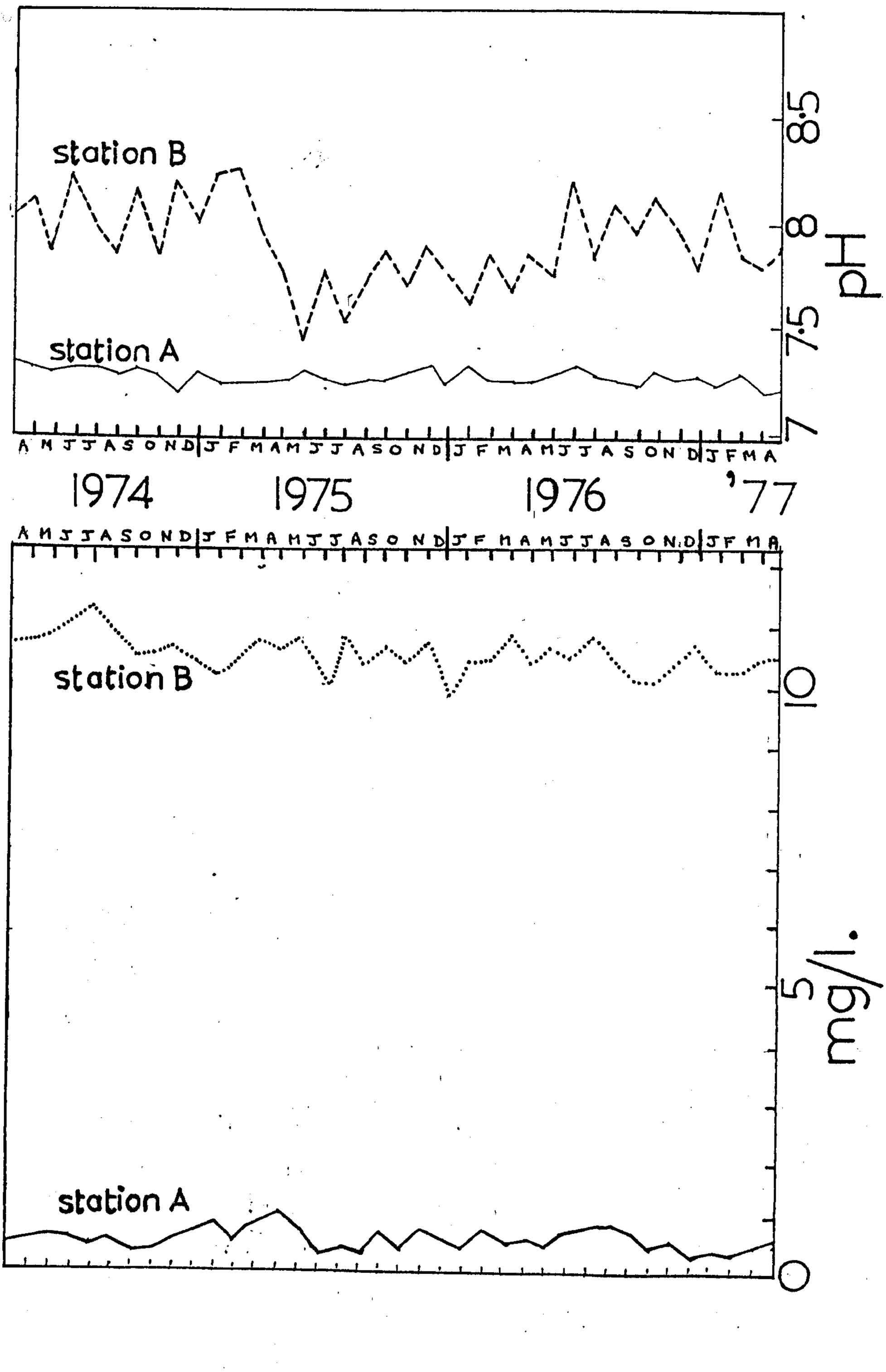


FIG. 5

Samples taken at station B also showed little variation, but, of course, were more or less fully saturated with oxygen, 10.68 ± 0.24 mg O_2/l). At no time did the pH of the spring deviate from 7.4. Station B samples usually gave higher readings of pH than station A, pH 7.8 ± 0.6). The full data is to be found in Appendix 2.

Although the temperature of water entering the pond at station A was found to be fairly constant throughout the year, there was as annual cycle of some 3°C variation around the mean of 9.5°C. The recordings from stations A and B are included in fig 6 together with the monthly mean air temperatures recorded at the Cromer station. The full data is to be found in Appendix 2.

The hydrographic survey revealed the Salts Hole to have a shallow margin of variable width which then sharply slopes down to a kidney-shaped depression just under 2m deep (fig.7). About 80% of the pond surface covers water whose depth is less than 2m deep and only a central depression of some 100 m^2 exceeds 2.5m deep. The maximum recorded depth was 2.7m.

The data recorded from the 59 stations of the survey consisting of salinity, oxygen content, pH, temperature and depth records is to be found in Appendix 3. Rather than discuss these generally, a transect running from NE to SW across the pond has been selected as representative. Its position is indicated in fig 7.

The results of the sieving analysis of substratum samples have been tabulated to include those factors which assist quantitatively in defining its nature. (Table 1). The first of these, the central tendency records the median diameter (Md) of the particles in the sample. It is determined at the point 50% frequency crosses the cumulative curve. Column a) of table 1 gives the phi value and column b) the value in mm of the median diameter. Despite the fact that this statistic gives only an average value for the sediment and cannot indicate the degree of spread of the data about this central tendency, it is clear that there are quite distinct groups of stations whose median diameters lie within the ranges of 2.4 - 4.4mm, 0.24 - 0.34mm, and 0.08 - 0.10mm. These stations could be qualitatively described as respectively gravelly, sandy and muddy. Only nine stations out of 59 fall outside these ranges.

The degree of scatter is represented by the quartile deviation (QDϕ), a statistic which records the number of phi units between the 25% and 75% points on the cumulative curve. A sediment with a small

Table 1 Substratum: sieving analysis

Sample	Central Tendency		Cumulative Curve		Quartile Deviation	Skewness
	Md ø	Md mm	Q_1 ø	Q_3 ø	QDø	Skø
A1	-2.07	4.40	-3.15	+0.80	1.98	+0.90
A2	-1.13	2.16	-2.82	+0.75	1.79	+0.09
A3	-1.04	2.10	-2.85	+0.74	1.80	+0.02
A4	+2.08	0.249	+0.43	+3.08	1.33	-0.33
B1	-2.00	4.00	-2.75	+0.85	1.80	+1.05
B2	-1.95	3.92	-3.00	-0.38	1.31	+0.26
B3	+0.73	0.63	-0.02	+1.25	0.64	-0.12
B4	+0.71	0.62	-0.05	+1.25	0.65	-0.11
B5	+2.13	0.246	+0.38	+2.95	1.29	-0.45
B6	+2.13	0.244	+0.38	+2.98	1.30	-0.45
C1	-1.85	3.70	-2.92	-1.10	0.91	-0.16
C2	-1.86	3.71	-2.47	-0.20	1.14	+0.53
C3	-1.49	2.91	-2.63	+0.86	1.75	+0.61
C4	+2.15	0.244	+0.35	+2.88	1.27	-0.54
C5	+1.97	3.98	+0.26	+2.95	1.35	-0.37
C6	+2.02	0.244	+0.30	+3.01	1.36	-0.37
C7	+3.60	0.084	+1.40	+5.70	2.15	-0.05
D1	-2.15	4.32	-3.00	+0.05	1.53	+0.68
D2	-1.46	2.90	-2.92	+0.67	1.80	+0.34
D3	+2.02	0.242	+0.05	+2.92	1.44	-0.54
D4	+1.86	0.316	+0.10	+2.92	1.41	-0.29
D5	+2.07	0.247	+0.32	+2.98	1.33	-0.42
D6	+1.90	0.271	+0.15	+2.88	1.37	-0.39
D7	+1.51	0.360	-0.15	+2.87	1.51	-0.15
D8	+3.52	0.090	+1.00	+5.40	2.20	-0.32

Table 1 Substratum: sieving analysis contd.

Sample	Central Tendency		Cumulative Curve		Quartile Deviation	Skewness
	Md Ø	Md mm	Q_1 Ø	Q_3 Ø	QDØ	SkqØ
E1	-1.42	2.89	-2.61	+0.66	1.65	+0.45
E2	-1.87	3.72	-3.05	+0.95	2.00	+0.82
E3	+2.05	0.246	+0.06	+2.98	1.41	-0.58
E4	+2.02	0.244	+0.31	+2.92	1.31	-0.41
E5	+2.10	0.247	+0.07	+2.95	1.44	-0.59
E6	-1.75	3.32	-2.58	+0.42	1.50	+0.67
E7	+1.98	0.252	-0.25	+2.90	1.58	-0.66
E8	+3.65	0.082	+1.25	+5.32	2.04	-0.37
F1	-1.42	2.94	-3.65	+0.61	2.13	-0.10
F2	+0.69	0.64	-0.11	+1.20	0.65	-0.15
F3	+0.69	0.64	-0.11	+1.28	0.70	-0.11
F4	-1.51	2.93	-2.63	+0.69	1.66	+0.54
F5	-1.31	2.55	-2.49	+0.82	1.66	+0.48
F6	-1.36	2.57	-2.49	+0.80	1.65	+0.51
F7	+2.05	0.246	+0.37	+2.94	1.29	-0.40
F8	+2.07	0.248	+0.31	+2.98	1.34	-0.43
G1	-1.49	2.91	-2.85	+0.65	1.75	+0.39
G2	+0.73	0.63	-0.08	+1.16	0.62	-0.19
G3	+0.74	0.62	-0.12	+1.14	0.51	-0.23
G4	-1.05	2.11	-2.85	+0.68	1.77	-0.04
G5	-1.40	2.70	-2.68	+0.54	1.61	-0.33
G6	+2.10	0.244	+0.35	+2.98	1.32	-0.44
G7	+1.84	0.317	+0.00	+2.91	1.46	-0.39
G8	+1.83	0.318	+0.01	+2.87	1.44	-0.40
H1	-2.12	4.40	-3.06	+0.01	1.54	+0.60

Table 1 Substratum: sieving analysis contd.

Sample	Central Tendency		Cumulative Curve		Quartile Deviation	Skewness
	Md ø	Md mm	Q_1 ø	Q_3 ø	QDø	Skqø
H2	+0.67	0.66	-0.37	+1.12	0.75	-0.30
H3	-0.71	0.63	-0.12	+1.25	0.66	-0.15
H4	-1.05	2.11	-2.37	+0.60	1.49	+0.17
H5	-1.32	2.55	-2.57	+0.63	1.60	+0.35
H6	+3.60	0.084	+1.50	+5.75	2.13	+0.03
J1	+2.06	0.247	+0.45	+2.93	1.24	-0.37
J2	+2.00	0.250	+0.23	+2.86	1.32	-0.46
J3	+2.20	0.220	+0.16	+2.96	1.40	-0.64
J4	+3.60	0.084	+1.45	+5.20	1.88	-0.28

SALTS HOLE: TEMPERATURE RECORDS

CROMER: MEAN MONTHLY MAXIMUM AND MINIMUM AIR TEMPERATURES

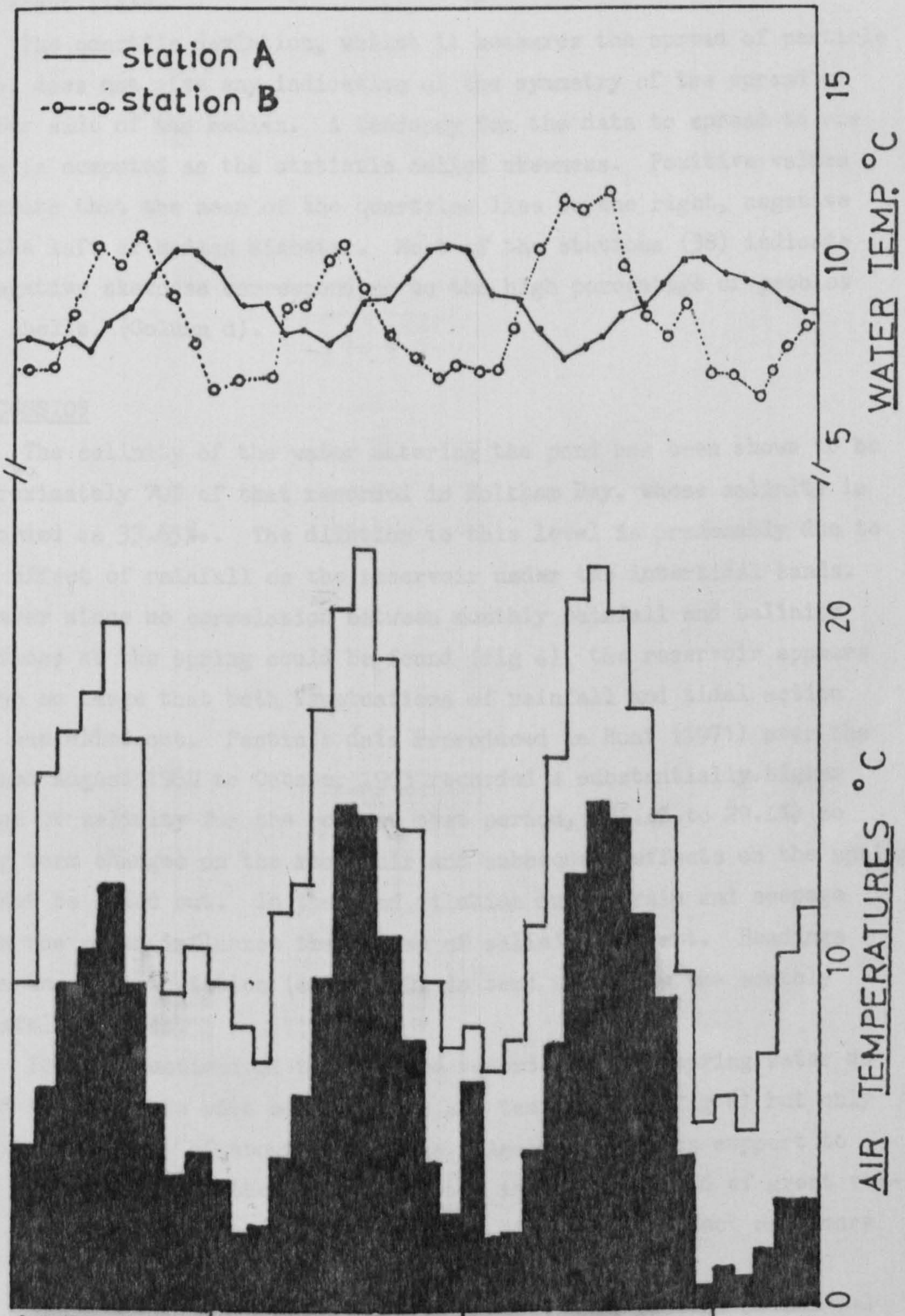


FIG.6

spread between the quartiles is regarded as being 'well sorted' and applies to those few where QDϕ is less than 0.7 (Column c). The majority of samples indicate that the sediment is not well sorted, that is to say that there is an irregular distribution of particles of different sizes.

The quartile deviation, whilst it measures the spread of particle size, does not give any indication of the symmetry of the spread on either side of the median. A tendency for the data to spread to one side is computed as the statistic called skewness. Positive values indicate that the mean of the quartiles lies to the right, negative to the left of median diameter. Most of the stations (38) indicate a negative skewness corresponding to the high percentage of pebbles and shells. (Column d).

DISCUSSION

The salinity of the water entering the pond has been shown to be approximately 70% of that recorded in Holkham Bay, whose salinity is recorded as 33.65‰. The dilution to this level is presumably due to the effect of rainfall on the reservoir under the intertidal sands. However since no correlation between monthly rainfall and salinity readings at the spring could be found (fig 4), the reservoir appears to be so large that both fluctuations of rainfall and tidal action are cancelled out. Pantin's data reproduced in Hunt (1971) over the period August 1962 to October 1963 recorded a substantially higher range of salinity for the pond at that period, (26.4‰ to 29.4‰) so long term changes on the reservoir and subsequent effects on the spring cannot be ruled out. In the pond, dilution due to rain and seepage from the marsh influence the degree of salinity present. Readings from the outlet station (station B) do tend to follow the monthly rainfall figures.

The fluctuations of temperature recorded in the spring water do tend to correlate with monthly mean air temperature (fig 6) but only after a time lag of about two months. Again this lends support to the contention that the water reservoir is both deep and of great volume. Water temperatures at the outlet, of course, reflect much more the ambient air temperature.

The pond is too shallow to develop a typical pattern of thermal stratification such as is found in dimictic freshwater lakes. The salinity of the water is frequently in excess of 24.7‰, at which it is most dense at its freezing point and therefore sinks. Also strong wind action or persistent heavy rain should both be sufficient to dis-

SALTS HOLE : DEPTH CONTOURS AND TRANSECT.

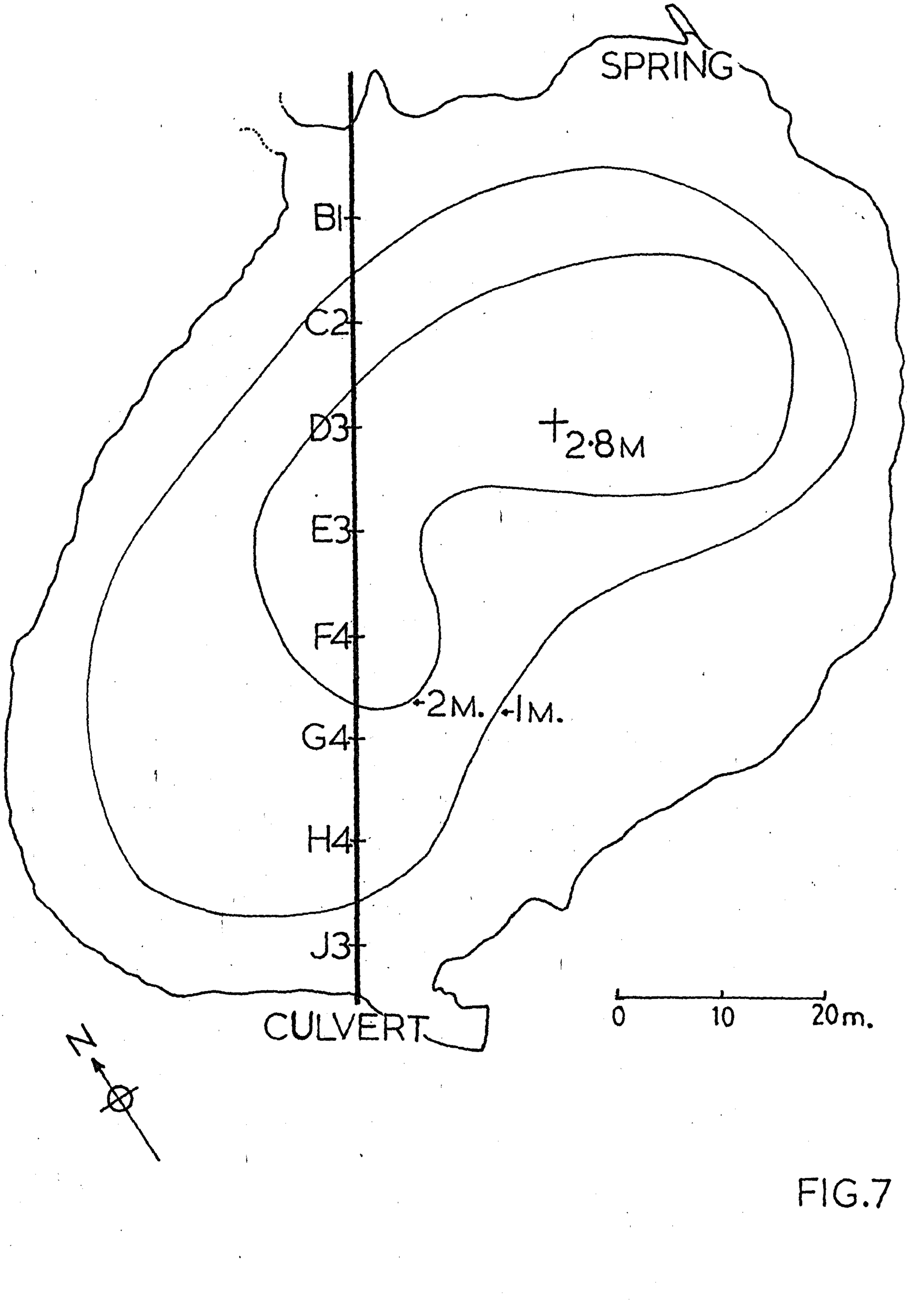


FIG.7

turb a developing thermocline. Notwithstanding, it may be seen from the data recorded in figs.8 and 9,both the March and October surveys do show a discontinuity in water temperature, where a cold core is covered by warmer waters. This pattern is repeated in the salinity concentrations recorded, for a halocline corresponding to the thermal strata is clearly present (figs 8 and 9). This salinity-temperature gradient appears to be maintained by the denser cold saline spring water flowing under water which has been diluted by rain or by freshwater draining into the pond from the surrounding marshes. This gradient may be overturned in periods of disturbed weather.

The cold lower stratum of the pond also maintains a reduced oxygen concentration in the vicinity of the anaerobic ingress of the pond water, but as this water flows across the pond, quite clearly a great deal of oxygen is absorbed, and the gradient is no longer discernable by station E6.

The weather was overcast in March, but sunny and warm in October and during the latter survey the surface layers gave consistently higher oxygen concentration readings than in March. The increased photosynthetic activity which was presumably taking place would explain this and also the higher pH recordings from the surface layers. These were the only departures from a pH range of 7.4 - 7.9 recorded from all stations.

All the physical conditions within the Salts Hole have remained remarkably constant during the five years in which they were monitored. This must be attributed to the nature of the saline spring and its reservoir, and to the influence it exerts in replenishing the waters of the pond. None of the lagoon and saline pond systems referred to earlier can claim such a degree of constancy and indeed it is difficult to imagine any brackish water habitats which are comparable except large bodies of water such as the Baltic Sea.

The pond is obviously non-tidal and wave action has never been sufficient to erode the margins nor damage their vegetation, during the period of monitoring.

The nature of the sediments of the Salts Hole may also reflect the negligible effects of wind and wave action on this area, since its establishment as a pond. The substrate is heterogeneous, with areas that could broadly be described as gravelly, fine-sandy and silty. They show discrete boundaries with little mixing. A fourth type substratum, coarse-sand, occurs in two small areas.(fig 10) This is particularly surprising as sand of this nature does not appear to be rep-

SALTS HOLE : TRANSECT MARCH 1975

a. TEMPERATURE

b. SALINITY

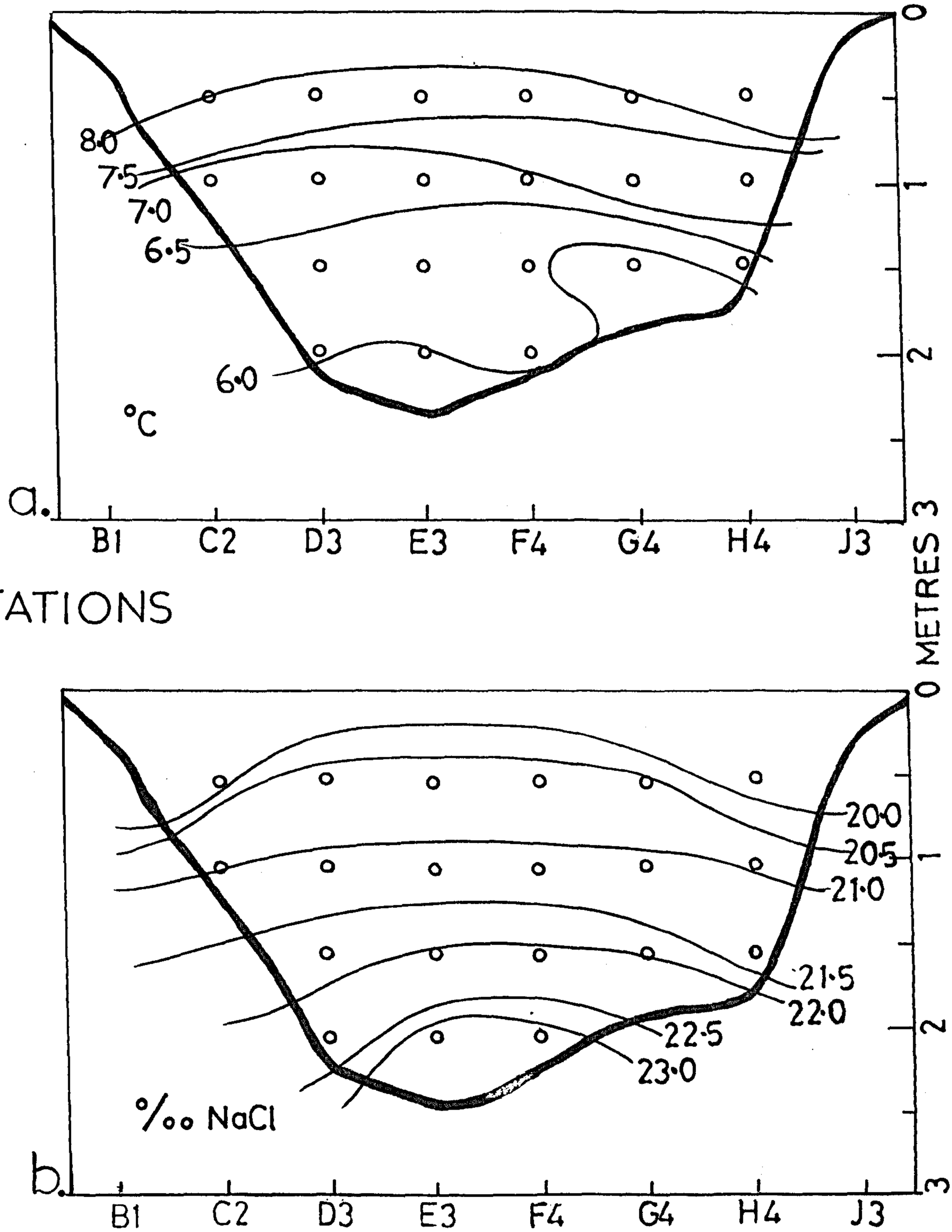


FIG.8

SALTS HOLE : TRANSECT OCTOBER 1975

a. TEMPERATURE

b. SALINITY

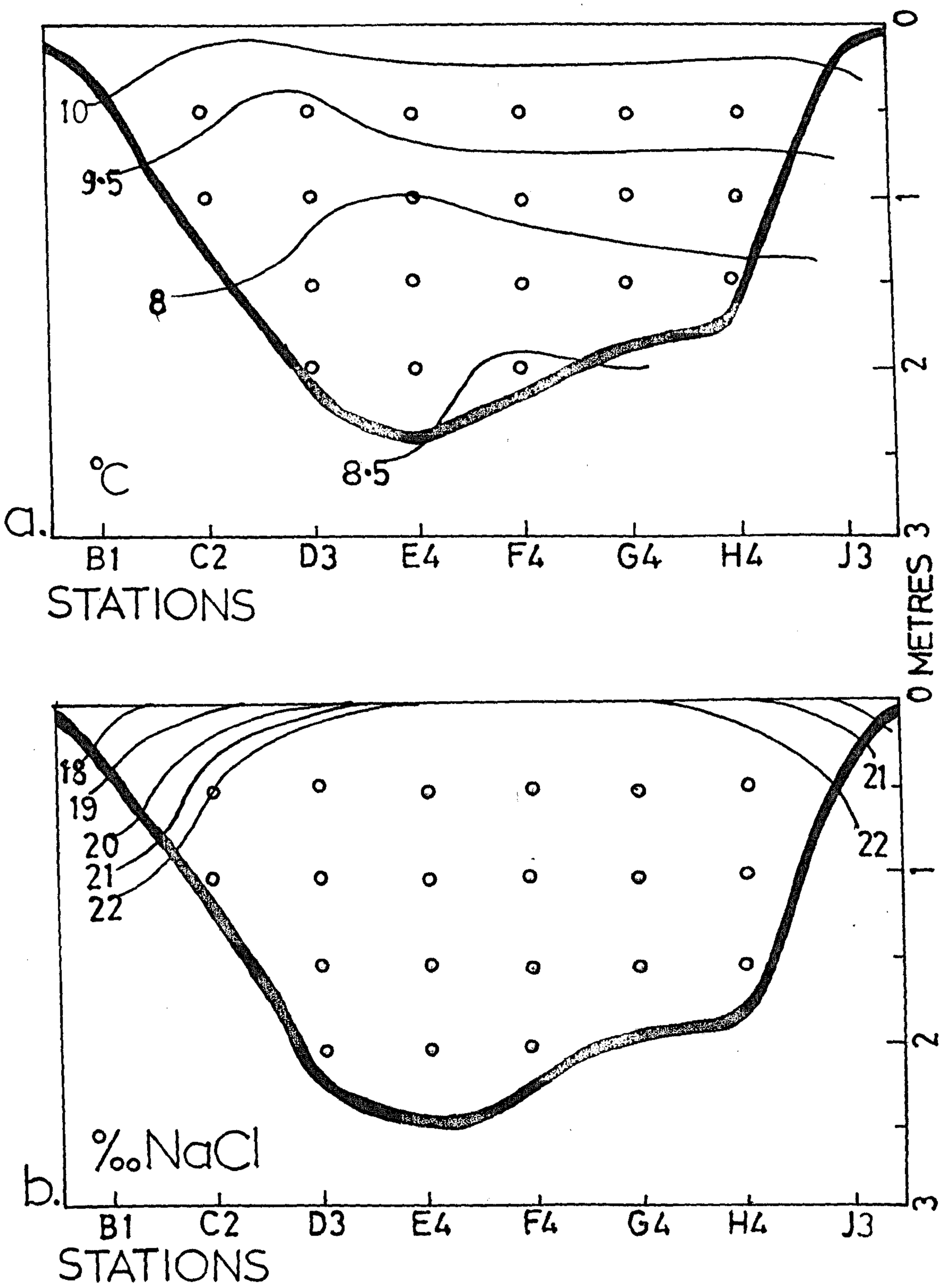


FIG.9

resented elsewhere in the immediate vicinity. The high quartile deviation recorded at 50 of the 59 stations clearly indicates that the sediment is very poorly sorted. Such a sediment is unlikely to have been laid down by wave action over an extended period of time. Indeed it gives substance to the suggestion that the Salts Hole may have been dug out in the early eighteenth century to act as a reservoir for salt water draining from the marshes. Comparable studies on samples taken from Holkham Bay and stabilised sand-dunes nearby show that they are both well sorted sediments. The asymmetrical distribution of particle size reflected in the data seems to be related partly to the high numbers of small pebbles present at many stations, and also to the shells of Cerastoderma glaucum (Poiret) which were also very frequently present.

SALTS HOLE:
NATURE OF SUBSTRATUM

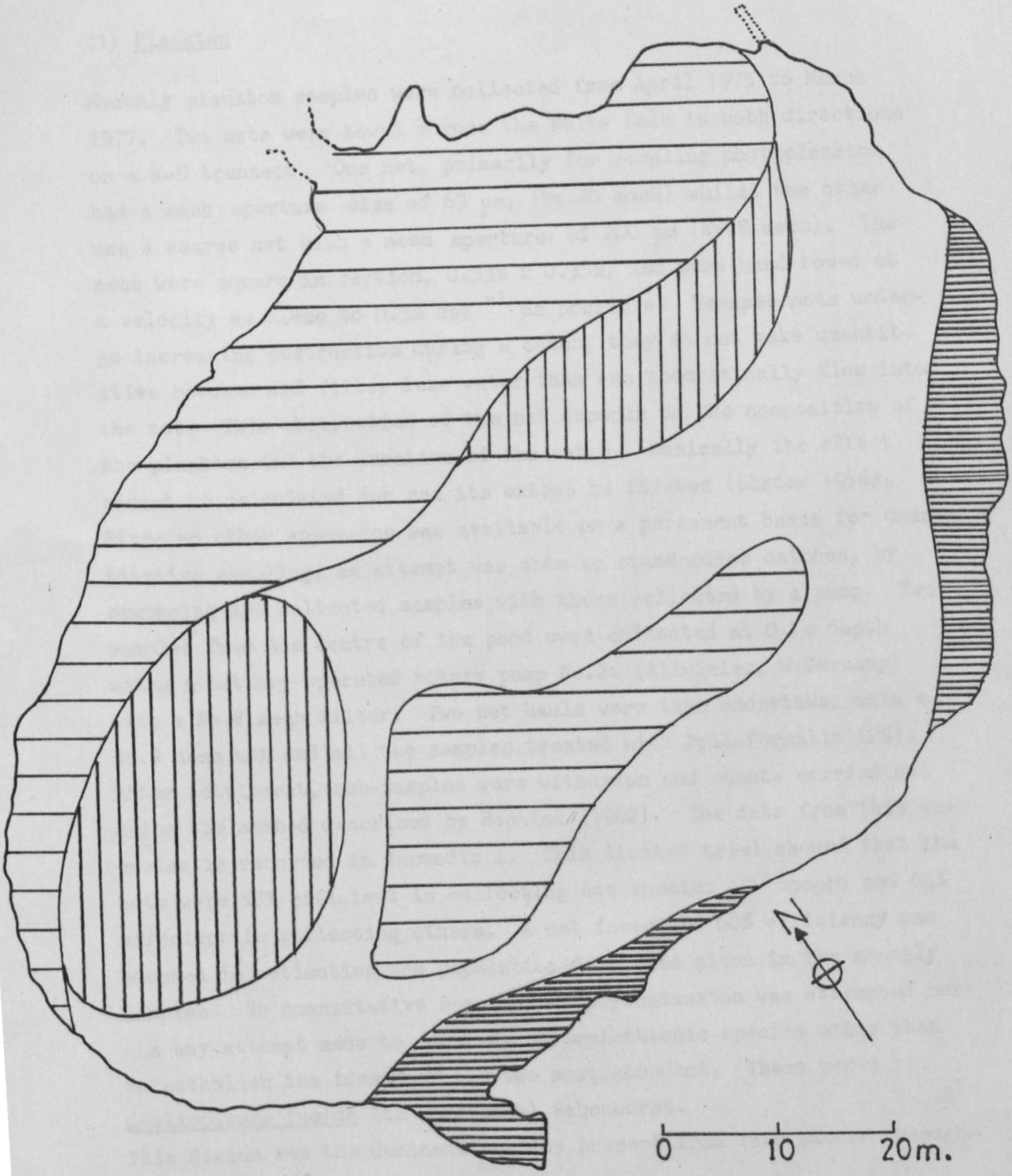


FIG.10

PLANT AND ANIMAL DISTRIBUTION.

(1) Plankton

Monthly plankton samples were collected from April 1975 to March 1977. Two nets were towed across the Salts Hole in both directions on a W-E transect. One net, primarily for sampling phytoplankton had a mesh aperture size of 63 µm, (No.20 mesh) whilst the other was a coarse net with a mesh aperture of 200 µm (No.8 mesh). The nets were square in section, 0.33m x 0.33m, and were hand towed at a velocity as close to 0.3m sec $^{-1}$ as possible. Because nets undergo increasing obstruction during a catch, they do not make quantitative catches and filter less water than can theoretically flow into the net. This obstruction of the net depends on the composition of the plankton and the duration of the catch. Basically the effect cannot be calculated nor can its extent be forseen (Elster 1958). Since no other apparatus was available on a permanent basis for quantitative sampling, an attempt was made to standardise catches, by comparing net collected samples with those collected by a pump. Two samples from the centre of the pond were collected at 0.1 m depth using a battery-operated rotary pump No.2. (Allwieler, W.Germany) with a No.8 mesh filter. Two net hauls were then undertaken with a No.8 mesh net and all the samples treated with Pril-formalin (2%). After settlement, sub-samples were withdrawn and counts carried out using the method described by Hopkins (1962). The data from this exercise is recorded in Appendix 4. This limited trial showed that the nets were 58% efficient in collecting one species of copepod and 64% efficient in collecting others. A net factor of 60% efficiency was adopted in estimating the population densities given in the monthly samples. No quantitative sampling of phytoplankton was attempted nor was any attempt made to identify phytoplanktonic species other than to establish the identity of the two most abundant. These were;

Scoliopleura tumida (de Brebisson) Rabenhorst.

This diatom was the dominant species present from late summer throughout winter until early spring.

Navicula digitoradiata (Gregory) Ralphs in Pritchard.

This diatom was also present throughout the year, but predominated during spring and early summer, in the samples examined.

Eight species of zooplankton were recorded in the coarse net samples, two of which were new records for the Salts Hole.

Acartia clausi Giesbrecht

This copepod was recorded in every sample collected. Although Gurney (1929) claims it is a species not normally associated with reduced salinity, in the Salts Hole it was always the most frequently recorded species.

Mesochra liljeborgi Boeck

The numbers recorded for this copepod fluctuated greatly. It was not recorded in the winter months of 1975-1976 or 1977-1978. It was taken in a towing in February 1974.

Praunus flexuosus (Muller)

Young specimens of the mysid were frequently taken.

Eurytemora velox (Lilljeborg)

This copepod has not been recorded previously, but is a typically euryhaline species which has been found in freshwater to hypersaline lagoons in Norfolk, (Gurney 1931).

Rathkea octopunctata (M.Sars)

This medusa is frequently taken in coastal samples and in habitats associated with reduced salinity. In the Salts Hole it was recorded for the first time in March 1978 and reappeared in samples in April 1979. The hydroid colony has not been found there despite an extensive search.

Cerastoderma glaucum (Poiret)

The veliger larva of this cockle was most frequently represented in the June samples when a maximum of $4x10^5/m^3$ were recorded.

Nereis diversicolor O.F.Muller

Larvae of the ragworm are not usually found in plankton samples, but in the Salts Hole a few were regularly found in early spring samples.

Pygospio elegans Claparede

The occasional larva of this polychaete was recorded in late spring samples.

As with all samples caught by plankton net, the number retained by this method may give a misleading impression of the actual density of the species recorded. Care was exercised in keeping the tow velocity low, however, and so the data has been included in fig 11 where as least monthly comparisions for the same species should be quantitative.

(2) Nekton and benthos

The following animals, all recorded in Hunt (1971) were regularly collected by netting during the period 1974 - 1979. No quantitative or

SALTS HOLE : MONTHLY PLANKTON SAMPLES

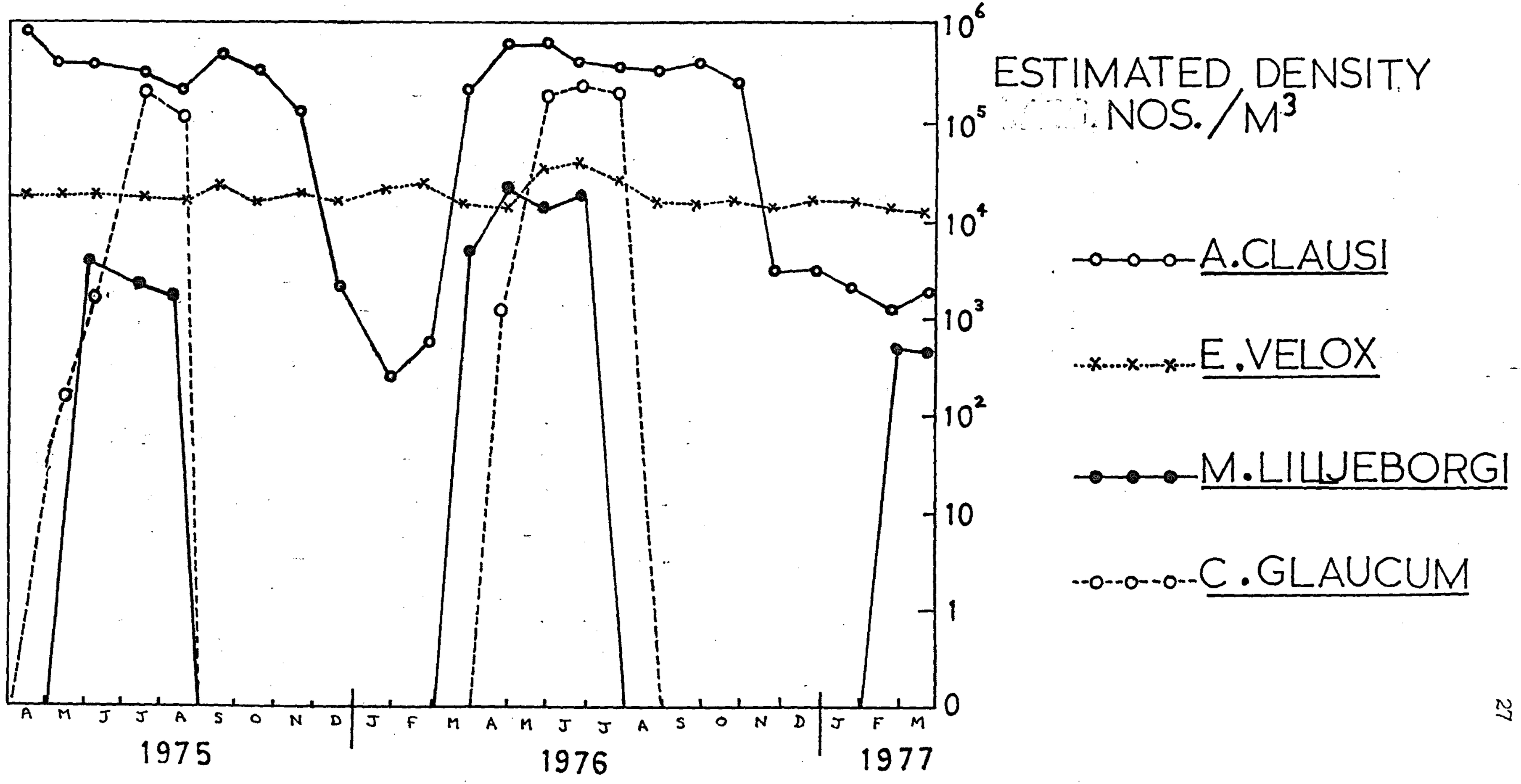


FIG.11

seasonal studies were carried out on these animals.

Cordylophora lacustris Allman
Sagartia troglodytes (Price) var. ornata (Holdsworth)
Lineus gesserensis O.F.Muller
Idotea chelipes (Pallas)
Gammarus duebeni Liljeborg
Microdeutopus gryllotalpa A. Costa
Paleomonetes varians (Leach)
Hydrobia ulvae (Pennant)
Gasterosteus aculeatus L.
Potamoschistus microps (Krøyer)

In addition to these species, two new records for species and positive identification of a third may now be given.

Coryne sp. The juvenile specimens of a species of corynid were found attached to stones dredged from the deepest part of the pond in March 1978. It was not possible to identify positively to which species they belonged.

Hydrobia ventrosa (Montague) This species is easily overlooked and was identified only in 1977. Its distribution in the pond is distinctly local, occurring along the western margin only, where it makes up approximately 3% of the hydrobid population.

Sphaeroma rugicauda Leach. A single specimen of a sphaeromid isopod was recorded by Hunt (1971) but lost before it could be identified. Presumably it was of this species as S. rugicauda has been recorded in the pond every year since 1974, although in surprisingly low numbers until 1978 when several hundred specimens were found attached to floating logs.

In addition to the macrobenthos collected in nets throughout the period indicated above, the substratum survey carried out in March 1975 provided an opportunity to examine the distribution of animals taken at the 59 stations of the 10 x 10m grid. There were seven species recorded here. They were the annelids Nereis diversicolor (O.F.Muller) Capitella capitata (Fabricius), Arenicola marina (L.), the molluscs Hydrobia ulvae (Pennant), Littorina 'saxatilis', Cerastoderma glaucum (Poiret) and the crustacean Corophium volutator (Pallas).
Examination of the 'saxatilis' winkle shows it to be of the form which Smith (1981) describes as Littorina rudis (Maton) and this is the name that has been adopted in this thesis.

Two subsamples, each of approximately 750cm^3, taken at each station, were hand sorted for macrobenthos. Table 2 records the numbers collected and the estimated density per m^2 for the seven species recorded.

Table 2

Macrobenthos collected during the Substratum Survey, March 1975.

Species	N	Density per m^2
Nereis diversicolor	59	10.02
Capitella capitata	10	1.69
Arenicola marina	22	3.73
Corophium volutator	35	5.93
Littorina rudis	61	10.34
Hydrobia ulvae	152	25.76
Cerastoderma glaucum	131	22.20

The complete data of numbers collected at each station may be found in Appendix 3.

Preliminary observations based on the presence and absence of the species collected suggested that some of them were discontinuously distributed in the pond. As may be seen in figs. 12 and 13, for example, neither A.marina nor H. ulvae were recorded from the central stations. To test the significance of these observations, the coefficients of multiple correlation were determined for variations in the nature of the substratum and in the depth of water.

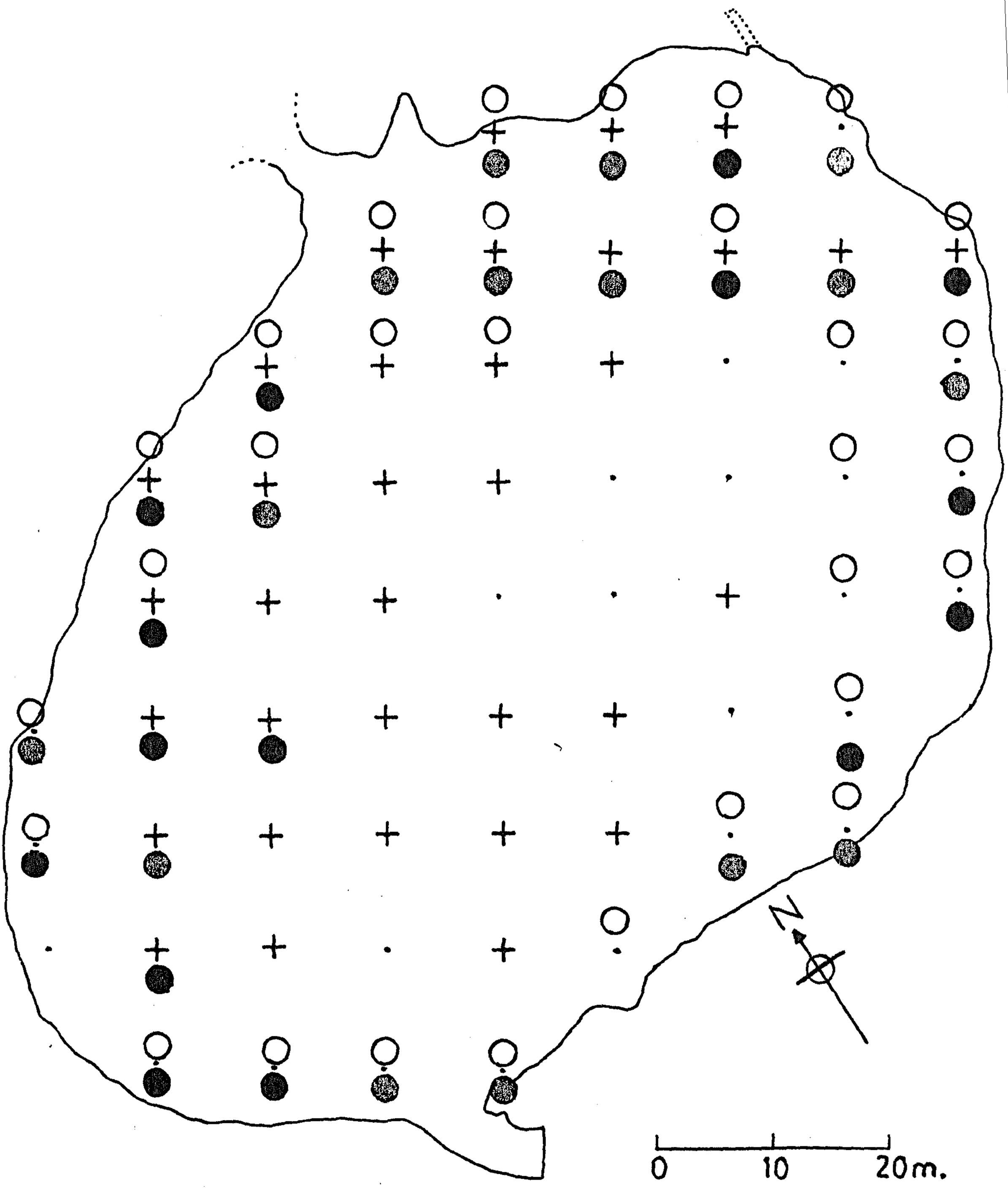
1) Correlation between substratum particle size and distribution.

The central tendency, (Mdϕ), was selected as the better expression of the nature of the substratum, for despite the poor assortment of particles, skewness, (Skqϕ), frequently used as an indicator, here deviated by 0.6 in only 8 of the 59 stations. This was the case because poor assortment is found in both particles which are smaller and larger than the median particle size, (Md). Each station was assigned to one of 13 catagories delineated in 0.5ϕ ranks from -2.5 to -2.0 ϕ (rank 1.), to 3.5 to 4.0 ϕ (rank 13). The number of stations for each rank may be found in Appendix 3.

The coefficient of multiple correlation between particle size and distribution was tested for each species present. The results are shown in table 3.

SALTS HOLE:

DISTRIBUTION OF MOLLUSCS



● HYDROBIA ULVAE

○ LITTORINA RUDIS

\+ CERASTODERMA GLAUCUM

FIG.12

Table 3

Salts Hole Macrobenthos: Correlation between distribution and substratum particle size.

Species	Multiple correlation coefficient	significance
Nereis diversicolor	0.266	2.021 +
Capitella capitata	1.82×10^{2}	0.135
Arenicola marina	-0.165	1.224
Corophium volutator	0.279	2.109 +
Littorina rudis	-4.07×10^{2}	0.302
Hydrobia ulvae	-9.72×10^{2}	0.716
Cerastoderma glaucum	-0.353	2.689 +

+ The critical value for a one-sided test at the 5% level = 1.64.

2) Correlation between water depth and distribution. The stations were assigned to one of six categories depending on the depth of water present from 0.5m or less in depth (rank 1), to 2.6m or more (rank6). The number of stations for each rank may be found in Appendix 3. The coefficients of multiple correlation were calculated and the results are shown in table 4.

Table 4

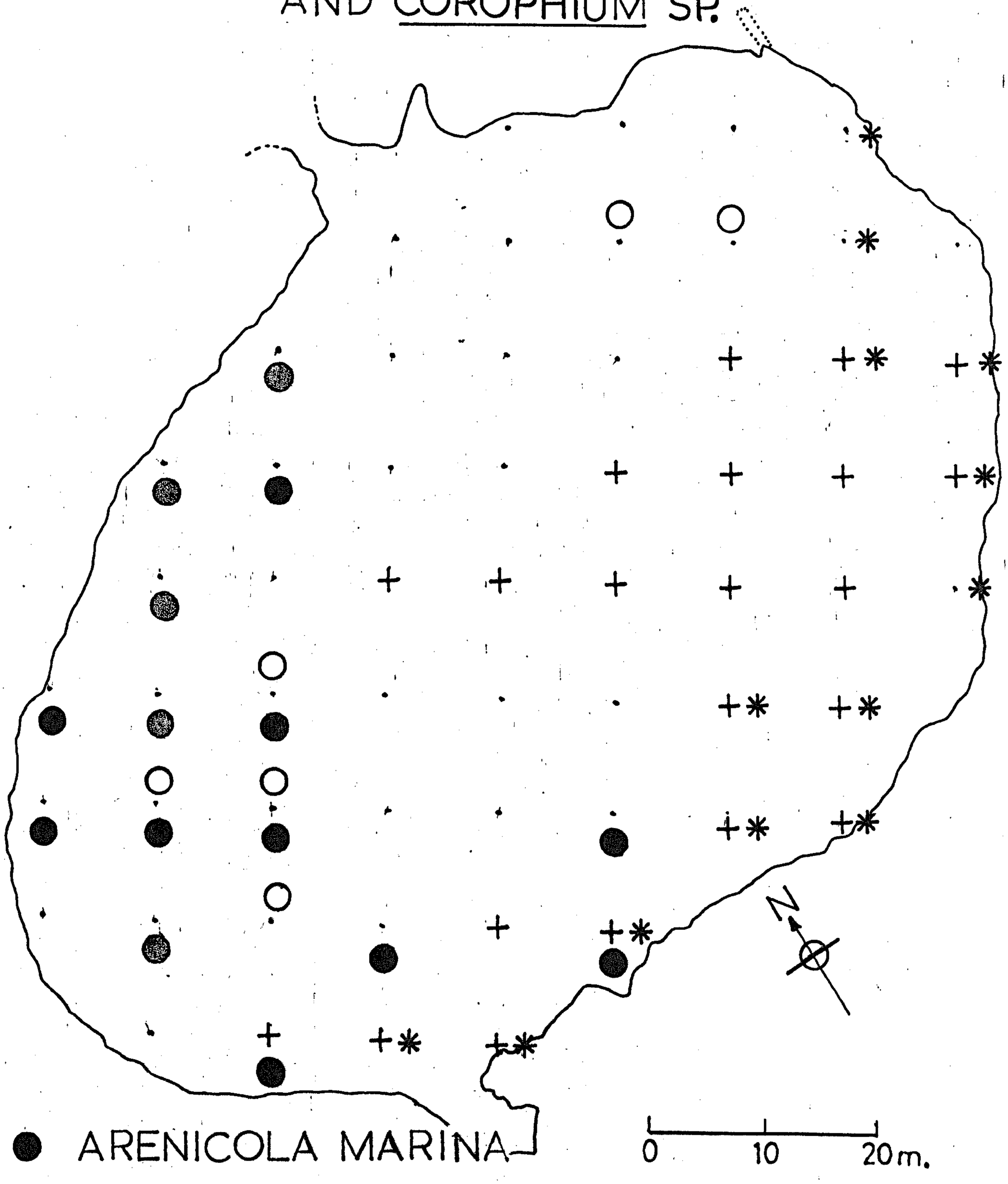
Salts Hole Macrobenthos: Correlation between distribution and water depth.

Species	Multiple correlation coefficient	significance
Nereis diversicolor	5.54×10^{2}	0.407
Capitella capitata	0.257	1.929 +
Arenicola marina	0.194	1.446
Corophium volutator	0.280	2.116 +
Littorina rudis	-0.498	4.054 ++
Hydrobia ulvae	0.437	3.250 ++
Cerastoderma glaucum	0.196	1.462

+ The critical value for a one-sided test at the 5% level = 1.64.

++ The critical value for a one-sided test at the 1% level = 3.14.

SALTS HOLE:
DISTRIBUTION OF ANNELIDS AND COROPHIUM SP.



● ARENICOLA MARINA
○ CAPITELLA CAPITATA
\+ NEREIS DIVERSICOLOR
* COROPHIUM VOLUTATOR

FIG.13

Estimation of Population size

During March and April 1977 an attempt was made to estimate the effective population size, ($\hat{N}e$ = estimated population of animals of reproductive age) for G.duebeni, I. chelipes and P. flexuosus.

The gammarids and isopods proved to be sufficiently immobile to collect from shallow water using a 0.33 m^2 net, placing it on the substratum and inserting a metal plate beneath. By gently moving the plate, both gammarids and isopods were dislodged from stones and were then hand-sorted from the weeds and detritus scooped up.

Subsequently, dredging was carried out to establish the presence or absence of the animals in deeper waters. No I. chelipes were taken at depths greater than one metre, but G. duebeni was found in all the dredge samples and was presumed to be distributed over the whole of the pond bed. The estimations of population size are given below in Table 6. The complete data may be found in Appendix 5.

Table 6

Estimated effective population size ($\hat{N}e$)

G. duebeni

mean number of adults /m^2	= 40.5
standard error	= 9.57
area of pond bed	= 5.2×10^3 m^2
$\hat{N}e$	= $2.10 \pm 0.50 \times 10^5$

I. chelipes

mean number of adults /m^2	= 50.58
standard error	= 8.41
area of pond bed less than 1m in depth	= 2.5×10^3 m^2
$\hat{N}e$	= $1.26 \pm 0.21 \times 10^5$

A population estimate on P. flexuosus was carried out in April using the Petersen method of capture, mark and recapture. One hundred and seventy five adult animals were marked on the telson with yellow 'Humbrol' enamel. They were dispersed in the pond after main-

taining them for 30 minutes in a tank, to ascertain that they all appeared healthy and active. Three days later, a recapture exercise was undertaken. Juveniles were excluded from the counts. The exercise was repeated in March 1979. The results are to be found in table 7.

Table 7

Estimated effective population size ($\hat{N}e$)

P. flexuosus

	Marked and released	Marked and recaptured	Unmarked
April 1977	175	4	1440
March 1979	104	4	1524

$\hat{N}e$ April = 63,175

$\hat{N}e$ March = 39,624

Discussion

The species diversity of diatoms in the phytoplankton was apparent in every sample taken. Hunt (1971) records 16 species which were identified in samples from the Salts Hole, but no attempt was made to establish whether they were still all present.

There were eight species of zooplankton recorded, of which three were sporadically present. R.octopunctata was observed only after regular monthly sampling had been discontinued. It was somewhat surprising therefore, in March 1978, to discover it present in appreciable numbers. An approximation of the maximal density was $3.1 \pm 0.5 \times 10^2/m^3$. The species is able to produce secondary medusae which could account for a rapid increase in numbers in a relatively short time. An extensive search for the hydroid colony was made in summer 1978, but without success. The medusae reappeared in March 1979.

The trochophore larvae of N. diversicolor, although able to swim well, are not planktonic and remain in the surface layers of the substratum. Nevertheless, a few larvae were caught in midwater in early spring in both years of the survey. Another annelid trochophore, thought to be the larva of P. elegans was present, on occasions, in mid-summer samples.

The veliger larva of C. glaucum was the predominant species during May and June, when populations of $5.2 \pm 0.4 \times 10^5/m^3$ were recorded. Figure 11 records the estimated population per m^3 for this and the remaining species during the two years in which monthly samples were taken. It has already been stated that the data is only an approximation, based on the sweep volume of the net, the number of animals caught and an estimate that 40% escape or do not enter a 200 µm net towed at 0.3m sec^{-1}. For some species this estimate was clearly unsatisfactory. Only young specimens of P. flexuosus were caught in the net for example presumably because the larger specimens could swim fast enough to avoid capture.

The permanent zooplankton is dominated by the three species of browsing copepods. A. clausi was most frequently represented. This species is essentially marine and is usually replaced by A.tonsa in conditions of reduced salinity, (Jefferies, 1962). Nevertheless the species is euryhaline and has been recorded from habitats where salinities varied from 10 - 40‰. It would be of great interest to know whether E. velox was overlooked in earlier sampling of the zooplankton, which seems unlikely, or whether it has appeared since Pantin's coll-

ections of the 1960's. E. velox is very frequently found in the adjacent salt-marsh pools and these must offer a possible channel of colonization. It seems to have become permanently established, and was still present in samples collected in 1981.

The other new records for species present are more easily explained. The corynid is clearly very easily overlooked, and,to present,no adult specimens have been found from which a positive identification to species level could be made. Similarly H. ventrosa may be easily mistaken for H. ulvae. Its presence in the Salts Hole shows that the species can live outside its previously reported salinity tolerances of 6 to 20‰ (Muus 1963). Within the above range, H. ventrosa usually replaces H. ulvae in areas rich in vegetation, and it is on the western margin of the Salts Hole where Chaetomorpha linum (Muller) fringes the pond that H. ventrosa is present. Only 3% of the hydrobid population here is H. ventrosa however. Its limited tolerance of higher salinities is one of several factors which may account for this. The correlation between the distribution of zoobenthic species and median particle size proved to be significant in three cases. N. diversicolor is known to be intolerant of gravelly substrata and C.volutator selects substrata which are composed of silt and fine sand, from which it can extract detritus. In suitable mud, populations of C. volutator can reach very high levels. Spooner and Moore (1940) recorded 1.1×10^4 animals per m^2. In the light of these figures, the population estimate for the pond of 2.0×10^2 per m^2 seems remarkably low, but is in fact of the same ordèr as populations recorded from sandy substrata in salt marshes, where the amount of detritus available for food is generally not very substantial. The positive correlation between the distribution of C. glaucum and particle size deserves comment. C. glaucum, unlike its marine counterpart C. edule (L), is frequently found on the surface amongst vegetation and in the Salts Hole it tends to accumulate in groups of about five or six bound together by byssus threads and sometimes interwoven with fronds of Ruppia cirrhosa (Petagna) Grande. It does not burrow into substrata which are predominately pebbly, although shells of dead cockles do not seem to present any obstacle. The cockles were clearly absent from those stations where the gravel content of the substratum was high. Of the remaining species examined H. ulvae and L. rudis, as non burrowers, would not have been expected to show significant correlation with type of substratum. This also

applies to C. capitata, for although this species is usually found in gravelly sand, this was not the case in two of the five stations in the pond where it was recorded. A. marina usually displays a very marked preference for silt or fine sandy substrates. It does not occupy these substrata in the Salts Hole, however, and is instead confined to the western margin where the gravel content is quite high. In similar conditions on the opposite side of the pond, it is not present. A. marina seldom exceeds 8cm in length in the Salts Hole, which may be taken as an indication that the substratum and its organic content are not ideal for maximal growth.

Correlation between water depth and distribution of zoobenthos is complicated by the variable nature of the substratum and was felt to be of limited reliability in defining the relative influence of this environmental parameter.

The presence/absence observations (fig 12) did indicate that both H. ulvae and L. rudis were limited to the margins of the pond - regardless of the nature of the substratum and this was supported by the corrlation statistic. Their concentration here may relate to the build-up of detritus around the pond margins, which is clearly observable. Both species migrate vertically in aquaria and attach near to the air/ water interface. The relatively few sites at which C. capitata was recorded were all at the same depth, but this species is not known to be sensitive in its submersion demands. C. volutator also appears to favour the parts of the ponds where water depth did not exceed one metre.(fig 13)

The estimates of effective population size (Nê) of the three species of crustaceans cannot be considered reliable. In the case of G. duebeni and I. chelipes it proved difficult to ascertain the efficiency of capture using the net directly applied to the substratum. It was a difficult operation to carry out, and there may have been escapes which, due to the stirring up of the substratum, were overlooked. Furthermore the absence of I. chelipes in the deeper trawls may indicate more about the relative inefficiency of the method than its restricted distribution. Similarly Petersen capture-recapture methods are known to be of limited value in many situations when they have been used. The most likely sources of error include the early death of the marked individuals due to the treatment, or by being more obviously recognised by predators. This may be especially true of a transparent animal like P. flexuosus. There is then the difficulty

of ensuring random distribution of the marked individuals in the pond. Notwithstanding this limitation, it was still felt worthwhile to include this data, because it is likely that they at least indicate the order of magnitude of the populations which are all,this being the case, small.

Physiological variation in Salts Hole species.

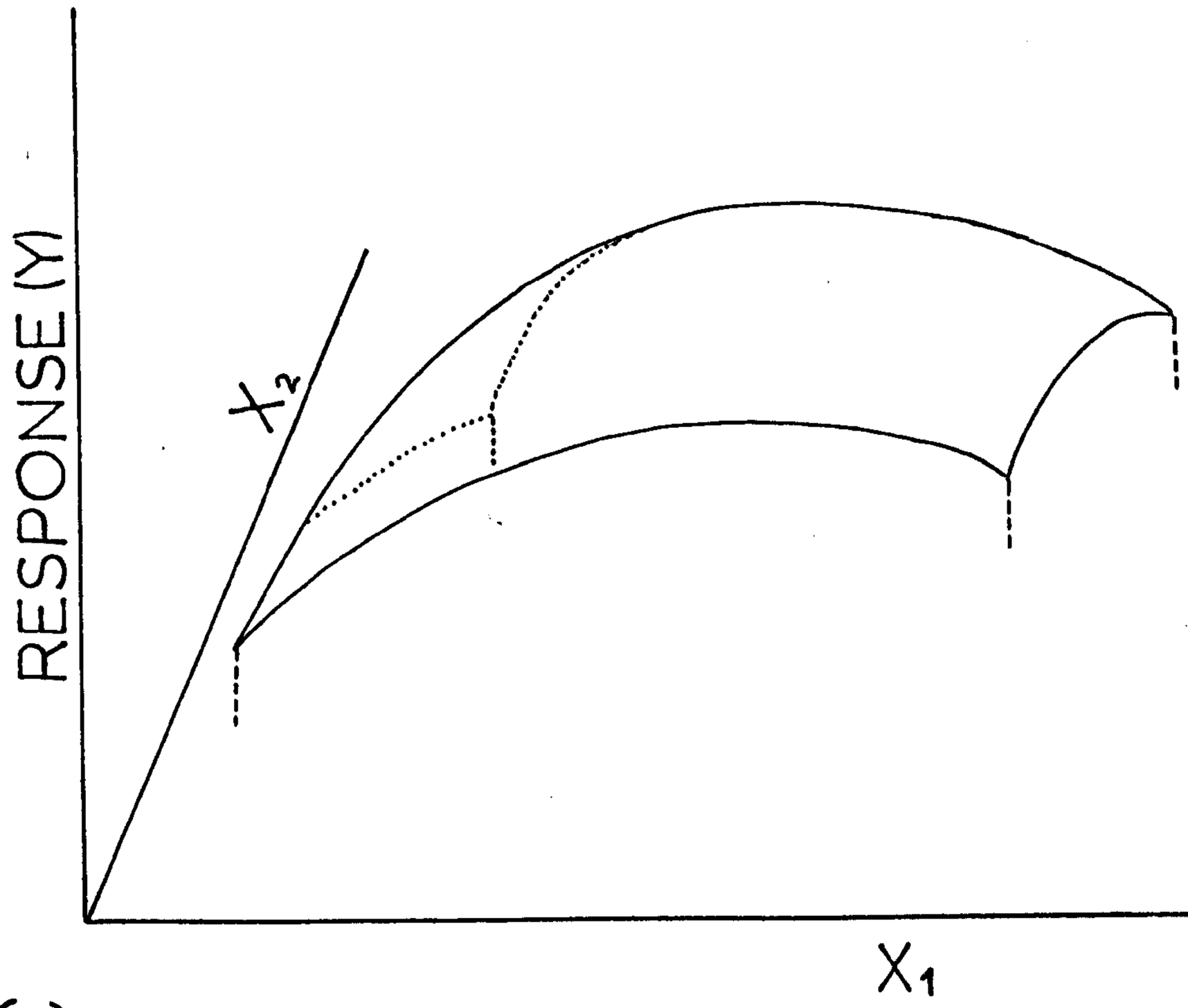
The combined effects of changes in salinity, temperature and oxygen concentration on the survival rates of selected species of crustaceans.

Introduction: multivariate response surfaces.

This investigation was designed to examine the nature and degree of genetic and environmental adaptation of representative species of the Salts Hole fauna. In view of the peculiar nature of conditions prevailing in the pond - that is to say - total immersion in tideless water where the salinity deviates little from 27‰ and where temperature and oxygen concentration remains relatively constant, the fauna is protected from many environmental extremes experienced by Holkham Bay populations. The survival rates of pond populations exposed to a wider range of temperatures, O_2 concentrations and salinities than they would experience in their natural habitat, were examined. The limits chosen were within the ranges usually tolerated by the Bay populations, however, since these were the populations from which the Salts Hole fauna was originally drawn. It was felt that any significant differences in mortality observed between the two populations, might shed light on the nature and degree of adaptation which has taken place during the time of their isolation from each other.

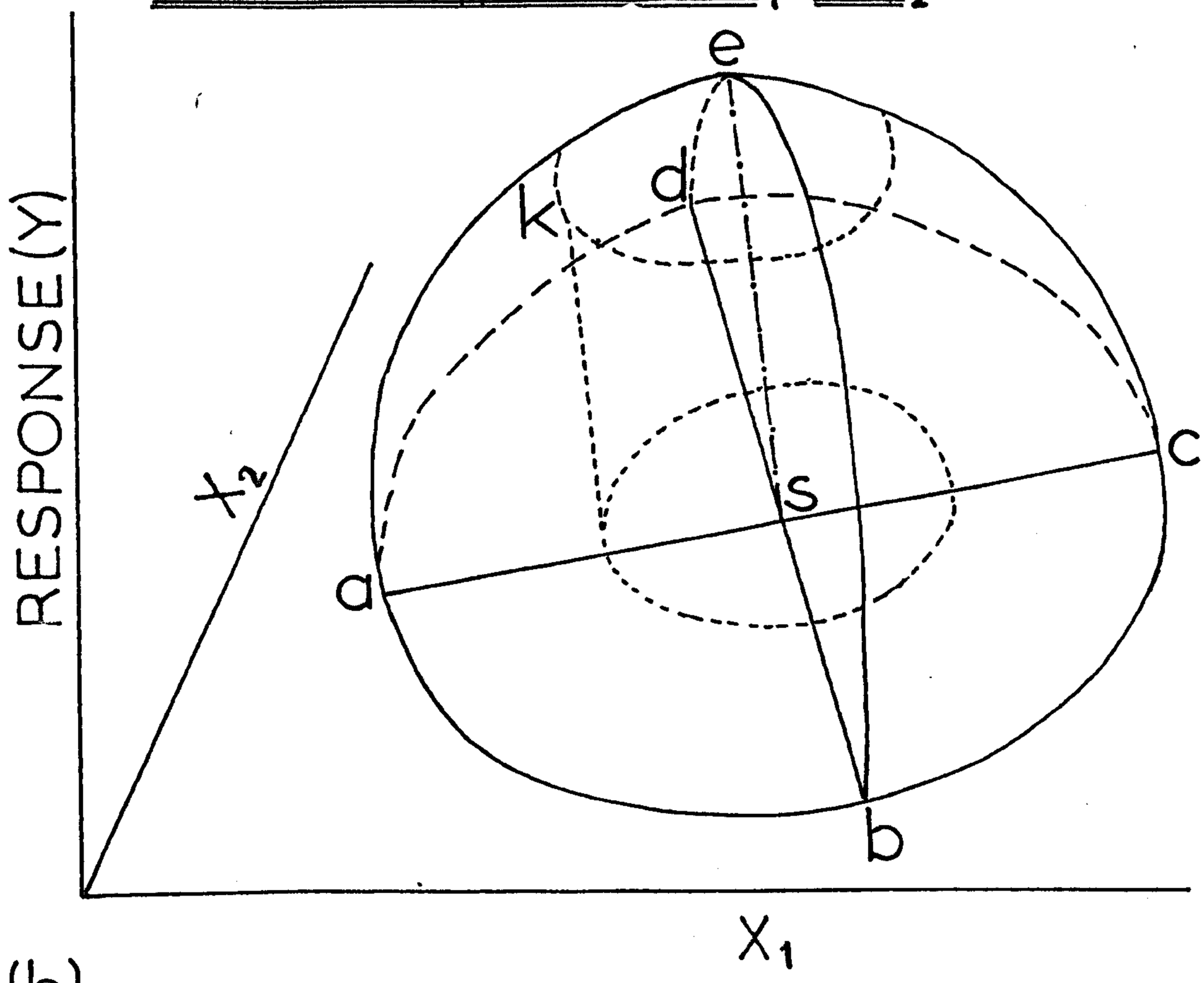
Kinne (1963) has shown that the biological effects of temperature, salinity and oxygen concentration are correlated in various ways. Temperature can modify the effects of salinity and enlarge, narrow or shift the salinity tolerance of an individual and salinity can modify the effects of temperature acclimation. The demand for oxygen increases in most species as temperature increases and salinity diminishes. Despite the acknowledged importance and interaction of these factors, studies involving their cumulative roles on tolerance and survival have been rather limited. This has in part been due to the difficulties of analysing complex phenomena but with the development of response surface methods by Box & Wilson (1951) and Peng (1967), it has become relatively easy to design experiments in which the effects of three environmental factors could be examined together.

Biological data, measured with respect to levels of two environmental variables tend to describe an arced plane in which there is some form of curvature in the relation between the magnitude of the response (Y) and the environmental variables, (x_1 and x_2). Simple curvature may be described by a second order expression such as



(a)

SECOND ORDER SURFACES BETWEEN RESPONSE, Y, AND TWO INDEPENDENT VARIABLES X_1 & X_2.



(b)

FIG.14

$$Y = b_o x_o + b_1 x_1 + b_2 x_2 + b_{11} x_1^2 + b_{22} x_2^2 + b_{12} x_1 x_2$$

where $b_o x_o$ is the value of Y for x_o

$b_1 x_1$ etc., represent the values of Y at x_1 x_2 x_1^2 etc., and are the regression coefficients. With the $x_1, x_2, x_1^2, x_2^2, x_1 x_2$ terms as independent variables, $b_o x_o$ measures the mean effect, $b_1 x_1$ and $b_2 x_2$ the linear effects and $b_{11} x_1^2$, $b_{22} x_2^2$ the quadratic effects relating to simple curvature. $b_{12} x_1 x_2$ is a second degree term measuring the linear x linear interaction between x_1 and x_2. If the curved plane is extended until the response is minimised at high and low levels of both variables x_1 and x_2, the nature of the surface generated is that generalized in fig 14. Where Y = 0, the curved plane meets the $x_1 x_2$ plane and describes an ellipse with axes ac and ab. The point of maximum response e on the surface, when projected onto the x_1, x_2 plane meets the plane at s, the centre of the ellipse. For purposes of visualisation, the second order surface in fig 14 may be considered as enclosing a solid. If the solid is lowered into a container of water the water will rise and wet the surface in a series of elliptical boundaries such as that upon which point k rests. If this ellipse is projected onto the $x_1 x_2$ plane, it will form an isopleth upon which response Y will take a specific value. Any number of isopleths could be described on the $x_1 x_2$ plane and associate a number of combinations of levels of the environmental variables with a specific level of response. If this projection is now repeated at differing levels of a third environmental variable, a three dimensional response surface is developed which encapsulates the response Y. It is in practice difficult to visualise this configuration and so in this study isopleths were designed from two variables, x_1 and x_2 at three levels of a third variable x_3, so that three cross-sections of the second order surface may be compared, (fig 15). Because biological response phenomena (eg survival, growth rate, oxygen consumption), often reach local maxima within a

ISOPLETHS OF TWO VARIABLES X_1 & X_2 AT 3 LEVELS OF A THIRD, X_3

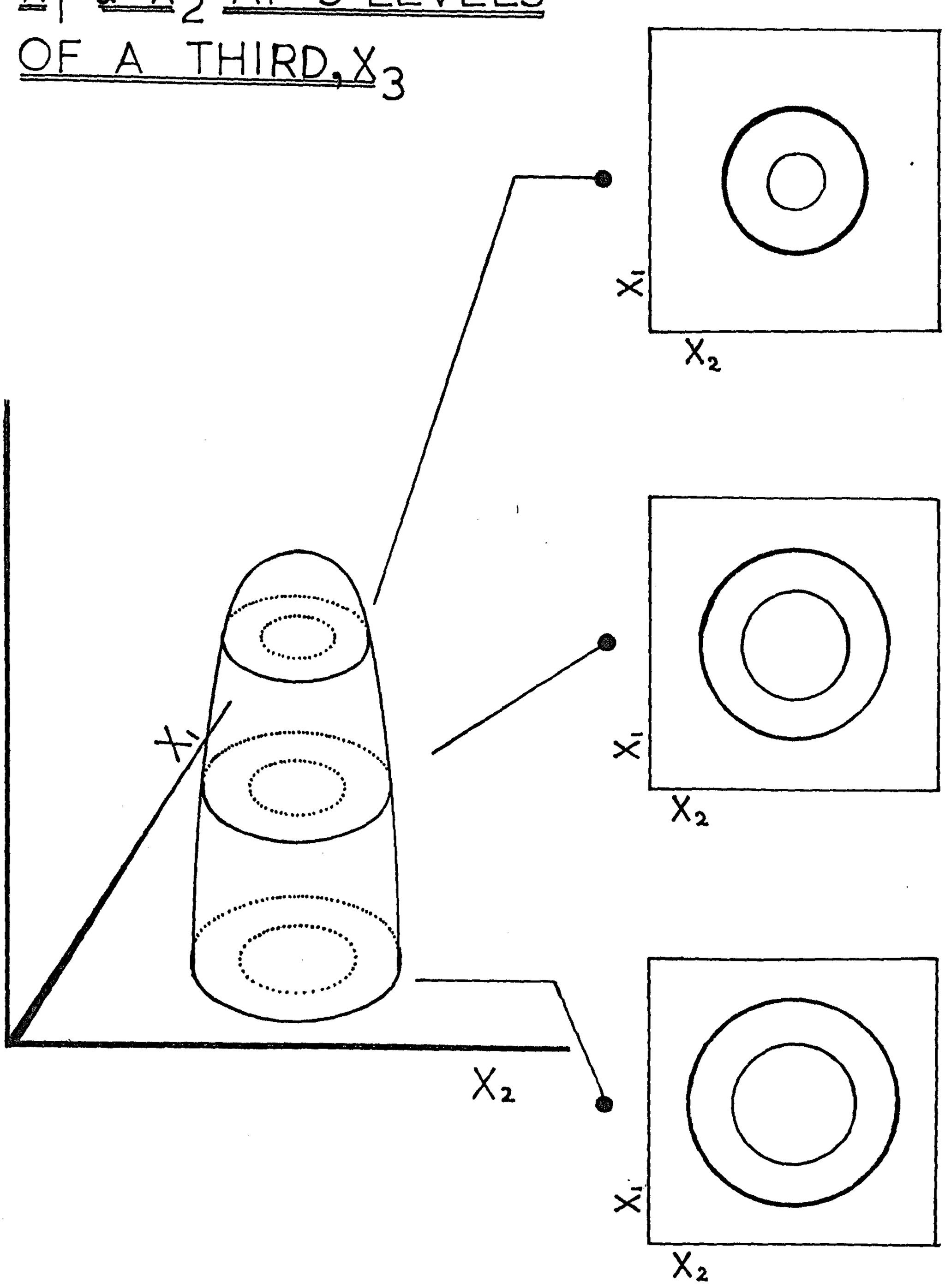


FIG.15

range of levels of an environmental variable, the second order expression provides a means of approximating the relationship between levels of environmental variables and response maxima.

The fitting of a response surface to the data provided by the effects of salinity, temperature and oxygen concentration on survival, was undertaken using standard regression analysis. Analysis of variance of data such as this usually suggests that a highly significant portion of the total variance is explained by treatment effects. The little that is not thought to be generated by departures of the true response surface from that generated with the empirical expression. Correcting this by employment of a cubic term (eg x_1^3), would place the present studies outside the range of experimental economy. As long as the factor space is limited to examining those levels of environmental variables found in the vicinity of response maxima, the fitting of these second order surfaces to experimental data remains a reliable method for approximating multivariable response relations.

The method of calculation of regression surfaces and on the guidelines employed in their interpretation are set out in Appendix 6.

Before the exploration of a multivariable response surface can be carried out, it is necessary to determine the range of the variables to be investigated. Initial trials which were carried out to establish these were designed to examine only the effects of a single environmental factor. This may seem misleading, especially when stress has been placed on the interaction of these factors. Interactions narrow responses rather than extend them however, and so by determining the limits of the response to a single variable, it could be safely assumed that the response centres for two or more variables would be encompassed.

a) The combined effects of salinity, temperature and oxygen concentration on adult I. chelipes.

Specimens of I. chelipes were collected from the Salts Hole on 14th March 1980 by washing samples of the alga Chaetomorpha linum Muller with pond water. The isopods which were identified using the key produced by Naylor (1975) are found in considerable numbers in the alga, and are easily removed. Other specimens were collected on several days during late March 1980 from Lodge Marshes, Holkham Bay (ref; TL452925). These were maintained separately from the Salts Hole animals, both being kept in aquaria in 25‰ SW which was fully oxygen-

ated and at 10°C. Hørlyck (1973) found that three days was sufficient for salinity acclimation in all the I. chelipes he studied. Nevertheless these animals were acclimated for at least seven days. Fronds of C. linum were added and the isopods grazed on the epiphytic flora associated with this alga.

Establishment of parameters: methods

Preliminary trials were performed exclusively on the Salts Hole population to determine the range of environmental parameters to be employed in calculating the response surfaces. Establishment of the salinity range was carried out by placing fifty animals in a series of eight 4.5 litre flasks containing 3 litres of SW from 5-40‰ intervals. The temperature of the tanks was 10± 0.5°C and air was passed through them for 30 minutes per 24 hours. Three trials of the experiment were performed, one in which all the animals were males, the second, in which they were all females and the last consisting of a mixed population of 25 males and 25 females. Specimens whose body length was less than 5mm were excluded. The number of animals surviving these treatments were recorded daily. Dead specimens were removed. A similar experiment was performed to establish the parameters of oxygen concentration. The procedure carried out was the same as that employed for the salinity determinations above except for the following modifications. The tanks were sealed and the SW was at a constant salinity of 20‰. This was flushed for 20 minutes with an appropriate mixture of nitrogen and oxygen to produce a range of oxygen concentrations from 2mg to 8 mg/l oxygen in 2mg/l stages. The number of animals surviving the treatments were recorded daily and the amount of O_2 remaining in the tanks monitored. Dead specimens were removed and the tanks were then reflushed with the appropriate gas mixture for 30 minutes.

Establishment of parameters: results.

Table 7 records the results of the preliminary experiment to establish salinity parameters and Table 8 presents the results of the experiment designed to locate parameters for oxygen concentration. The complete data which is summarised in the above tables may be found in Appendix 7.

At 5‰, nearly 90% mortality was noted even after 24 hours ex-

Salinity ‰	Mean % survival and standard deviation after											
	24 hours		48 hours		72 hours		96 hours		120 hours		144 hours	
5	10.67	3.40	8.67	1.88	3.33	1.88	3.33	1.98	2.00	2.00	0.00	-
10	70.67	3.78	68.00	4.90	59.33	4.10	58.00	4.32	55.33	5.78	52.00	6.54
15	70.00	3.26	67.33	3.40	58.00	4.26	55.33	4.16	53.33	4.12	52.67	4.98
20	96.67	0.94	95.33	0.94	80.67	4.10	79.33	3.40	76.67	3.40	76.00	3.26
25	98.67	1.88	97.33	0.94	85.33	3.78	84.67	3.40	83.33	5.26	81.33	4.12
30	98.00	2.82	95.33	3.40	82.00	2.84	74.67	6.18	74.00	7.12	72.67	6.18
35	96.00	3.26	94.00	2.82	80.00	5.88	68.67	6.80	60.67	10.88	52.67	6.74
40	42.00	9.54	33.33	4.10	18.67	4.72	8.67	3.76	3.33	2.80	2.00	2.00

Table 8 I. chelipes % survival to a range of salinities

O_2 conc. mg/l	Mean % survival and standard deviation after											
	24 hours		48 hours		72 hours		96 hours		120 hours		144 hours	
8	98.67	1.86	96.00	0.00	94.00	1.62	88.67	1.88	86.67	3.20	84.67	3.26
6	98.00	1.62	96.00	2.82	94.67	2.49	94.00	3.26	90.67	4.10	84.67	2.48
4	96.00	3.26	93.33	2.48	92.00	1.62	90.00	3.26	84.67	3.20	78.67	4.70
2	74.00	5.88	60.00	2.32	38.00	14.89	24.67	5.70	22.67	5.70	22.67	5.72

Table 9 I. chelipes % survival in response to a range of O_2 concentrations.

posure and by 144 hours no animals were left alive (Table 7). Over 50% survived exposure to 10‰ salinity for 144 hours, however. On this evidence, it was decided to set the lowest salinity for the response surface experiment at 10‰ since this was very near the threshhold for adaptation. At levels of salinity in excess of 35‰, a similar survival pattern with 80% mortality recorded after 72 hours. The upper limit for the response surface was accordingly chosen at 30‰. Since the middle value must be equidistant from the highest and lowest value, 20‰ was established for this. In the preliminary oxygen concentration experiment (Table 8) no significant difference was demonstrable between the 8mg/l and 6mg/l levels, but thereafter higher levels of mortality were demonstrable. The upper limit for oxygen concentration was therefore chosen to be 8mg/l. Mortality at 2mg/l steadily increased from 40% after 48 hours to nearly 80% after 96 hours. This level was therefore chosen as the lowest value for the response surface. A middle value of 5mg/l was thus established. An annual temperature variation of only a few degrees, with a mean of 9.5°C has been recorded in the spring water entering the pond and although the water in the pond itself shows a somewhat less restricted range than this, it is still narrow compared to conditions in the Bay itself. It was considered of limited value to carry out experimentation on the pond animals to establish temperature coordinates. Consequently these parameters were arbitarily chosen to include the lowest and highest water temperatures which I. chelipes could be anticipated to be subjected to in the pond. These were 5°C and 15°C, giving a middle value of 10°C. It is important because of the design of the experiment not to choose a timescale where mortality reaches 100% for any of the parameters recorded separately. This is approached at 120 hours for high salinities. It was decided to run the response surface experiment for 96 hours. This was 24 hours more than the maximum time Hørlyck (1973) found necessary for salinity acclimation, but within the limits imposed by the design.

A Kruskal-Wallis test was performed on the data for both salinity and oxygen concentration to establish whether the medians of the three replicate samples were similar. This proved to be significant at the 5% level in both cases, thus establishing that sex differences do not appear to be influential in determining mortality under the conditions of the experiments.

Determination of response surface. Method.

The animals were acclimated for at least 14 days in water of 20‰ salinity, aerated for 30 minutes every 24 hours and kept at 10°C. This corresponds to the method described previously except that during the acclimation and throughout the experiment the animals were kept constantly illuminated with a striplight giving a surface illumination of 1.5×10^3 lux. This was decided to be an appropriate refinement since Janssen and Kallander (1968) have shown that in I. chelipes activity increases in low light intensities.

Fifty animals, 7mm or more in length were placed in 4.5 litre flasks containing 3 litres of SW of the appropriate salinity. Temperatures were maintained by thermostatically controlled water baths. Mixtures of oxygen and nitrogen gases were bubbled through the water for 20 minutes and the flasks were then sealed. Every day the number of surviving isopods was recorded, the dead specimens removed, and a further aeration with the appropriate gas mixture carried out. In each pair of containers one sample consisted of Salts Hole animals, the other from Bay animals. Pairs of containers were set up for concentrations of 2, 5 and 8 mg/l O_2, at 10, 20 and 30‰ salinity, 9 pairs of containers in total. The first experimental run was started on 30.4.80 at 5°C and then-on the following dates, 6.5.80 at 10°C, and 15.5.80 at 15°C. A replicate series of experiments were then repeated at 5°C (18.5.80), 10°C (24.5.80) and 15°C (30.5.80). A surface illumination of 1.5×10^3 lux was maintained throughout the experiment.

Determination of response surface. Results.

A full account of the methods used to interpret the dynamic properties of response surfaces is given in Appendix 6. Briefly, four features are observable.

1) Euryplasticity and Stenoplasticity

It is difficult to interpret these terms quantitatively, but Precht et al (1966) suggests that an animal might be considered euryplastic if 90% or more of maximum response (eg. survival) were possible over 50% of the normally available range of the environmental variable considered. This may be deduced from examining the area covered by the 90% survival contour.

2) Interactions

Rotation of the X axes of the surfaces imply the presence of an interaction between the two environmental variables acting on the response.

3) Changes in tolerance or resistance

The centre of the surface, hence maximum resistance may move with respect to the axes of measurement, in this case to changed O_2 concentration.

4) Changes in capacity

These are observed by changes in the absolute value of the response surface centres.

Immersion in the experimental conditions for 96 hours just encompassed the total response (ie. 0-100% mortality). The percentage survival for each combination of environmental variables is given in Table 10. The anovar data of this experiment may be found in Appendix 8.

The equations of the response surfaces produced were of the following form;

$$\hat{Y} = b_o x_o + b_1 x_1 + b_2 x_2 + b_{11} x_1^2 + b_{22} x_2^2 + b_{12} x_1 x_2$$

The calculated equations are:

SALTS HOLE.

O_2 concentration = 2mg/l

$$\hat{Y} = 56.61 - 8.56x_1 + 9.67x_2 - 18.17x_1^2 - 23.06x_2^2 + 0.75x_1x_2$$

with standard errors of

56.61 ± 2.80, -8.56 ± 2.06, 9.67 ± 2.06
-18.17 ± 3.57, -23.06 ± 3.57, 0.75 ± 2.52

O_2 concentration = 5mg/l

$$\hat{Y} = 67.32 - 10.02x_1 + 10.70x_2 - 21.52x_1^2 - 19.03x_2^2 - 2.82x_1x_2$$

with standard errors of

67.32 ± 4.41, -10.02 ± 3.24, 10.70 ± 3.24
-21.52 ± 5.62, -19.03 ± 5.62, -2.82 ± 3.97

O_2 concentration = 8mg/l

$$\hat{Y} = 64.89 - 8.96x_1 + 8.87x_2 - 11.42x_1^2 - 8.33x_2^2 - 5.05x_1x_2$$

with standard errors of

64.89 ± 3.73, -8.96 ± 2.74 8.87 ± 2.74
-11.42 ± 4.75, -8.83 ± 4.75 -5.05 ± 3.36

HOLKHAM BAY

O_2 concentration = 2mg/l

$$\hat{Y} = 53.13 - 19.08x_1 + 20.38x_2 - 12.36x_1^2 - 17.49x_2^2 - 6.18x_1x_2$$

with standard errors of

53.13 ± 4.62, -19.08 ± 2.53, 20.38 ± 2.53
-12.36 ± 4.38, -17.49 ± 4.38, -6.18 ± 3.10

O_2 concentration 5mg/l

$\hat{Y} = 55.30-17.01x_1+20.43x_2-25.35x_1^2-12.33x_2^2-11.62x_1x_2$

with standard errors of

55.30 ± 9.17, -17.01 ± 5.02, 20.43 ± 5.02
-25.35 ± 8.69, -12.33 ± 8.69, -11.62 ± 6.15

O_2 concentration 8mg/l

$\hat{Y} = 63.70-14.40x_1+11.01x_2-21.69x_1^2-9.14x_2^2-3.87x_1x_2$

with standard errors of

63.70 ± 5.94, -14.40 ± 3.25, 11.01 ± 3.25
-21.69 ± 5.64, -9.14 ± 5.64, -3.87 ± 3.99

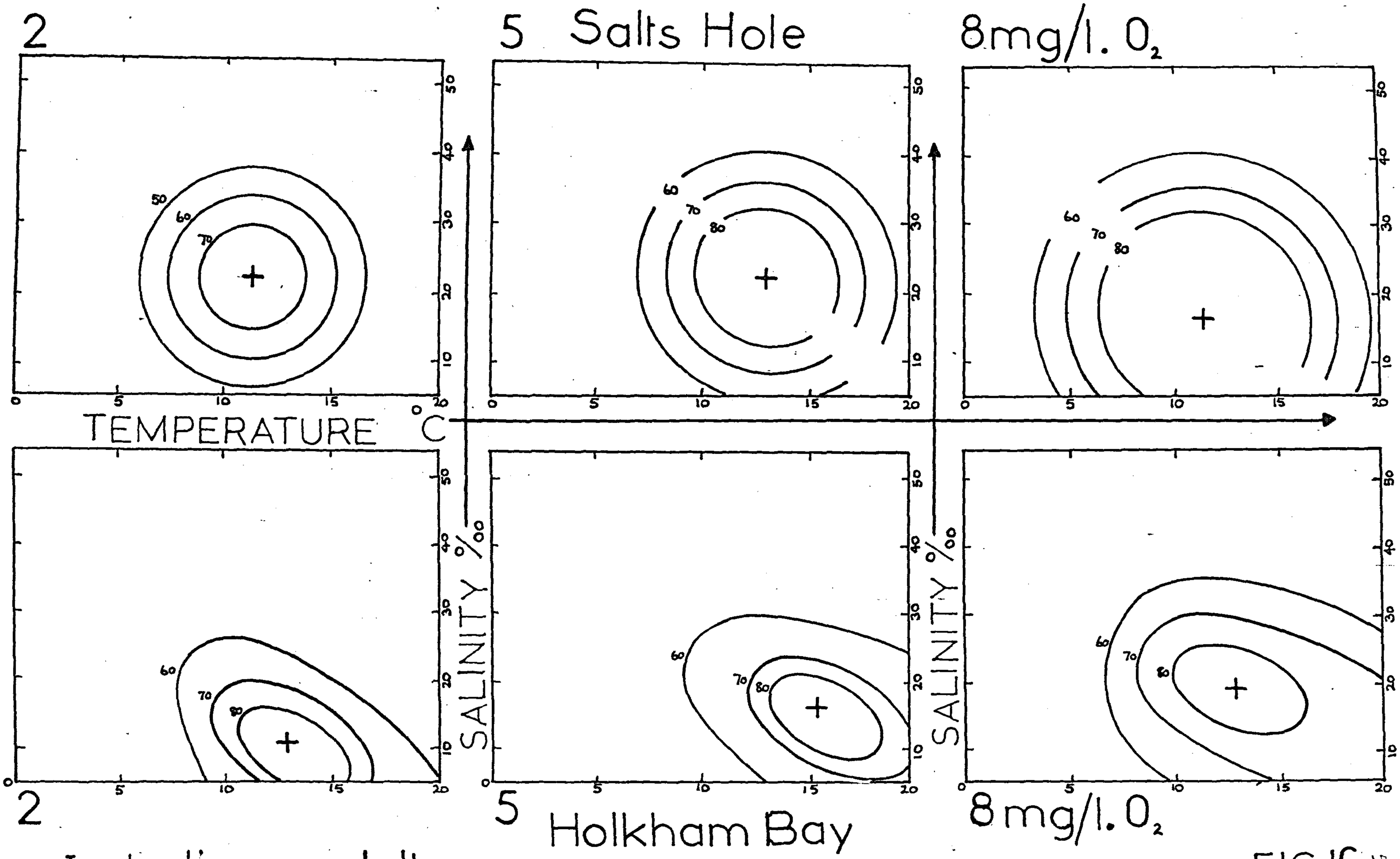
where $\hat{Y}$ = Estimated response in angular units, x_1 = temperature and x_2 = salinity.

The fitted response surfaces show that survival was unaffected by oxygen concentration except in the case of the Salts Hole samples at 2 mg/l, where mortalities were increased by 10% compared to the Bay samples (fig.16). Jones (1974) has shown that I. chelipes maintains a constant respiratory rate in relation to salinity changes from 7 to 35 ‰ at 8°C, and that this rate is low compared to crustaceans of similar size. (0.85 - 1.13 ul. O_2/mg dry wt/hour).

The change in position of the response surface centre especially in the Bay samples indicates the O_2 concentrations are having some effect on the animals which seem less able to tolerate higher salinities when the O_2 concentration is low. This phenomenon is not obvious in the Salts Hole samples where there is an overall increased tolerance to higher salinities (fig.17).

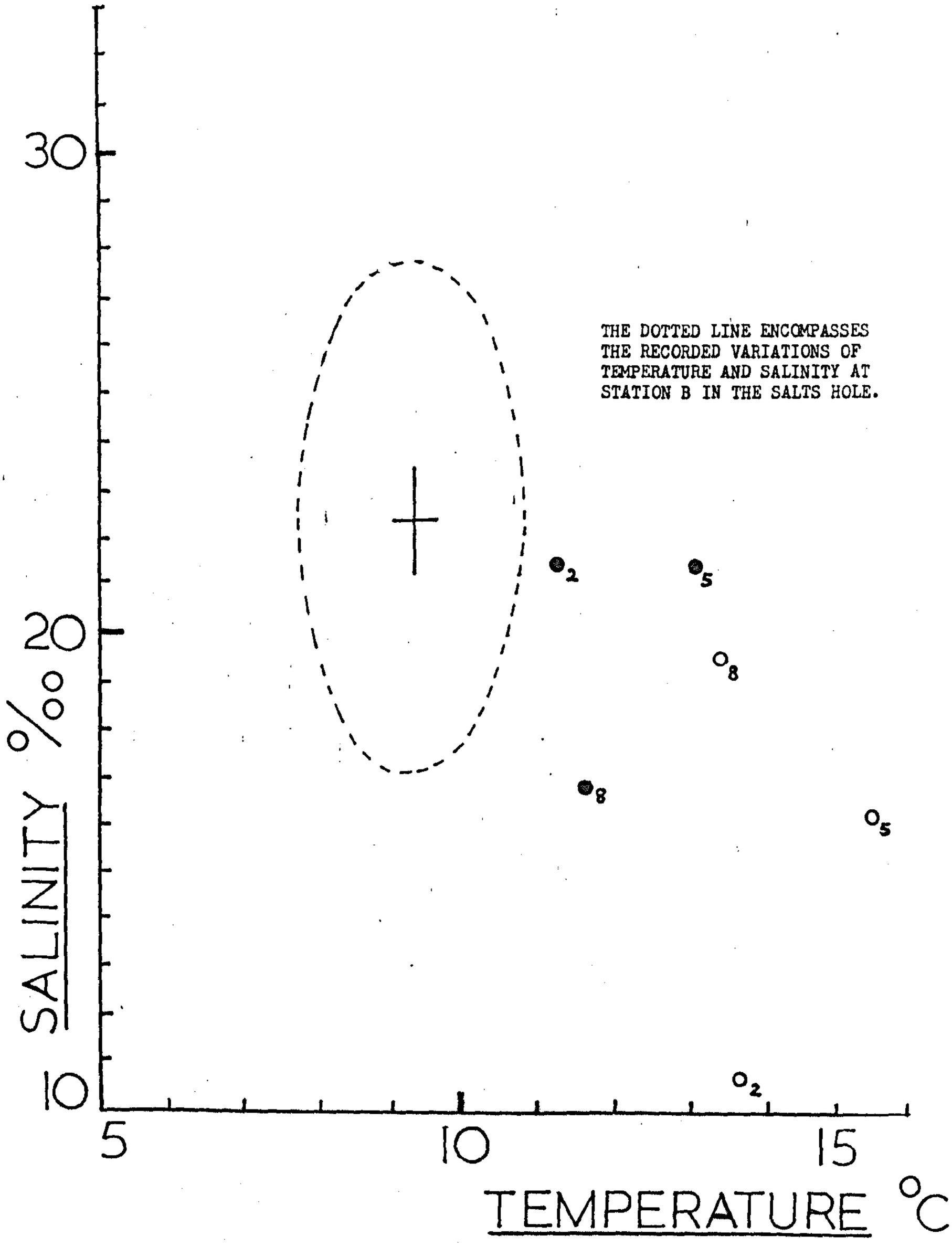
The rotation of the axes in all three of the Bay axes, when compared to the Salts Hole samples indicates that in the former strong interaction between salinity and temperature effects is taking place. Increasing temperatures lead to increased sensitivity to higher salinities. This was not the case in the Salts Hole samples where minimal interaction was recorded.

These noticeable differences in tolerance equate well with observations of the variability of this species in the field. Salemaa (1979) states physiological adaptability is a prominent feature of



I. chelipes, adult

FIG.16



THE RESPONSE SURFACE CENTRES OF THE SALTS HOLE SAMPLES ARE INDICATED BY ● .THOSE OF THE BAY SAMPLES BY ○.
THE NUMBERS 2,5,and 8 REFER TO THE OXYGEN CONCENTRATION IN mg/l.

I.chelipes : RESPONSE SURFACE CENTRES

FIG.17

I. chelipes. Remarkable differences have been found in life cycles, ecological roles, physiological responses and external morphology of local Idotea populations from different seas. In the face of this variation, I. chelipes may be expected to show greater degrees of euryplasticity than are evident in this study. For an estuarine species, the fitted response surfaces are quite restricted. The tolerance to lower temperatures (5°C and below) is suspect, as well as to higher salinities. I. chelipes shows an interesting behavioural response to salinities approaching 30‰ in nature. Howes (1939) describes how they will crawl out of the water onto a mudbank, until the salinity falls. This behaviour may indicate how Idotea survives the extremes of the salinity range to which it is exposed in estuaries. In the experimental tanks it was not possible for the species to leave the water.

Table 10

Idotea chelipes. Percentage survival of adults for immersion of 96 hours in 9 salinity temperature combinations at oxygen concentrations

Salts Hole Specimens

Trial	O_2 conc mg/l	5°C Salinity 10	20	30 ‰	10°C Salinity 10	20	30 ‰	15°C Salinity 10	20	30 ‰
1	2	10	56	40	10	90	34	0	16	18
2		2	42	30	16	88	22	0	4	18
mean		6	49	35	13	89	28	0	10	18
1	5	14	74	56	46	92	64	4	28	32
2		8	72	66	38	88	60	2	18	20
mean		11	73	61	42	90	62	3	23	26
1	8	28	90	86	66	84	72	24	32	52
2		36	86	82	60	76	84	36	46	48
mean		32	88	84	63	80	78	30	39	50

Holkham Bay Specimens

Trial	O_2 conc mg/l	5°C Salinity 10	20	30 ‰	10°C Salinity 10	20	30 ‰	15°C Salinity 10	20	30 ‰
1	2	8	82	80	0	70	74	0	4	16
2		6	88	76	2	78	74	0	0	12
mean		7	85	78	1	74	74	0	2	14

Table 10 continued

Trial	O_2 conc mg/l	5°C Salinity 10	20	30 ‰	10°C Salinity 10	20	30 ‰	15°C Salinity 10	20	30 ‰
1	5	0	68	80	6	74	80	0	0	8
2		0	68	72	10	78	82	0	0	4
mean		0	68	76	8	76	81	0	0	6
1	8	22	56	12	82	86	16	68	90	30
2		30	40	6	84	80	10	80	82	22
mean		26	48	9	83	83	13	74	86	26

b) The combined effects of salinity, temperature and oxygen concentration on juvenile I. chelipes.

Despite several attempts to establish a breeding colony of I. chelipes in the laboratory, only limited production of juveniles from Salts Hole samples could be obtained and none from the Bay samples. This study was consequently discontinued.

c) The combined effects of salinity, temperature and oxygen concentration on adult Gammarus duebeni.

Specimens of G.duebeni were collected from the Salts Hole on 14th April, 1980 by wading with a push net. A collection from the shallow water east of Wells Harbour, (928438), in late April, constituted the Bay samples and were maintained independently from the Salts Hole animals. Both samples were kept in aquaria in 20‰SW and oxygenated for 30 minutes every 24 hours. The level of salinity is appreciably lower than that experienced by the Salts Hole fauna. Attempts to acclimate the Bay specimens at 25‰led to quite high mortalities at first. It was found that 20‰, although much higher than the salinity at which G. duebeni was collected in Wells Harbour, (8-12‰) proved to be satisfactory for both populations. They acclimated to this without appreciable mortalities during the first 14 days. The species was identified using the key designed and lent by Kolding (1981). Every 14 days the water in the tanks was changed. Bulnheim (1972) showed this was necessary to provide an adequate supply of microflora for the gammarids to feed on. Juveniles were readily produced in both aquaria. Kinne (1962) found that five days was sufficient for salinity acclimation and Haywood (1970) established that even 2-3 days was adequate for acclimation to conditions within the range encompassed in

their natural habitat. It was found convenient to acclimate these animals for at least 7 days in the stock tanks before experimentation. A similar experiment to that performed on I. chelipes and described on page 44, was carried out to determine the range of salinities to be employed in calculating the response surfaces.

Fifty animals from the Salts Hole stock were placed in a series of nine 4.5 litre tanks containing 3 litres of SW which ranged from 0-40‰ in 5‰ intervals. The temperature of the tanks was maintained at 10°C ± 0.5°C and oxygen was bubbled through them for 30 minutes every 24 hours. Three trials of the experiment were performed, all males, all females and a mixed population. Specimens whose body length was less than 5 mm were excluded. The number of animals surviving these treatments were recorded daily. Dead speciemns were removed. It was decided for ease of operation to maintain the oxygen concentrations and temperatures at the same levels as those which were employed for I. chelipes ie. 2, 5 and 8 mg/l O_2 and 5, 10 and 15°C.

Results

Table 11 records the results of the preliminary experiment to establigh salinity parameters. The full data may be found in Appendix 7. A Kruskal-Wallis test was performed on this data to determine whether the medians of the three replicate samples were similar. This proved to be significant at the 5% level, thus establishing that, under the conditions of the experiment sex differences do not influence survival. Several of the mortalities appeared to be related to moulting activity. Lockwood and Inman (1973) showed apparent permeability doubles at ecdysis and remains at a higher level for several days, and so this was to be expected. Exclusion of all animals below 5 mm meant that very few moults were recorded.

Very few mortalities were recorded over the whole range of salinities tested. Even after six days over 70% survival was found from 10‰ to 40‰. The levels chosen for the response surfaces were therefore arbitary and were based on the 80% survival level. The length of run was thought to be critical as Bulnheim (1972) has shown that O_2 consumption declines by as much as 15% when the animals fast. Five days seemed to be the minimum time in which clear responses could be recorded, before this factor became critical. At this time 80% survival was recorded at 10‰ and 30‰. These were chosen as the lower and upper parameters with a central value of 20‰.

Salinity‰	Mean % survival and standard deviation after											
	24 hours		48 hours		72 hours		96 hours		120 hours		144 hours	
5	89.33	4.11	86.00	4.98	80.67	3.39	75.33	2.49	71.33	1.89	68.67	0.94
10	87.33	2.49	84.67	3.39	82.67	4.11	80.00	4.89	80.00	4.89	78.67	4.22
15	96.67	3.39	96.67	3.39	95.33	3.77	94.00	2.83	92.67	2.49	92.00	2.83
20	96.67	3.39	96.00	3.27	94.00	4.90	91.33	2.49	90.00	1.63	89.33	2.49
25	98.00	1.63	97.33	2.49	96.00	2.83	95.33	2.49	94.00	1.63	93.33	3.13
30	95.33	3.39	92.00	1.63	91.33	2.49	84.66	3.77	80.00	4.90	76.67	2.49
35	94.67	4.11	90.67	1.89	86.00	4.32	81.33	3.69	79.33	4.11	77.33	5.25
40	91.33	2.49	88.00	5.89	83.33	3.77	78.00	4.32	74.66	4.71	70.66	2.49

Table 11 G. duebeni % survival in response to a range of salinities.

Determination of response surfaces: Method

The animals were acclimated for seven days or more in SW at 20‰, oxygenated for 30 minutes every 24 hours, and maintained at 10^{o}C. Constant illumination at 1.5×10^{3} lux was maintained by striplight. Fifty animals 5 mm or more in length were placed in 4.5 litre containers with three litres of water of the appropriate salinity. Temperatures were maintained within $\pm$ 0.5^{o}C by thermostatically controlled water baths. Mixtures of O_2 and N_2 were bubbled through the containers for 30 minutes, the oxygen concentration checked and the flasks were then sealed. Every day the number of surviving amphipods was recorded, the dead specimens removed and a further aeration with the appropriate gas mixture carried out. A surface illumination of 1.5×10^{3} lux was maintained. In each pair of containers one sample consisted of Salts Hole specimens, the other from Bay specimens. Pairs of containers were set up for concentrations of 2, 5 and 8 mg/l O_2 at 10, 20 and 30‰ salinity, 9 pairs of containers in total. The first experimental run was started on 5.6.80 at 5^{o}C and then on the following dates, 10^{o}C (11.6.80) and 15^{o}C (17.6.80). A replicate series of experiments was then repeated at 5^{o}C (23.6.80), 10^{o}C (30.6.80) and 15^{o}C (6.7.80).

Determination of response surfaces: Results.

The percentage survival for each combination of environmental variables is given in Table 12. The equations of the response surfaces produced were of the following form;

$$\hat{Y} = b_o x_o + b_1 x_1 + b_2 x_2 + b_{11} x_1^2 + b_{22} x_2^2 + b_{12} x_1 x_2$$

The calculated equations are;

Salts Hole

O_2 concentration 2 mg/l

$$\hat{Y} = 71.91 + 0.05x_1 - 0.44x_2 - 2.56x_1^2 - 9.43x_2^2 + 0.33x_1x_2$$

with standard errors of

71.91 ± 3.52, 0.05 ± 1.92, -0.44 ± 1.92
$- 2.56 \pm 3.34$, -9.43 ± 3.34, -0.33 ± 4.72

O_2 concentration 5 mg/l

$$\hat{Y} = 84.11 - 2.35x_1 + 0.39x_2 - 8.29x_1^2 - 9.38x_2^2 - 1.13x_1x_2$$

with standard errors of

$84.11 \pm 1.63, -2.35 \pm 0.89, 0.39 \pm 0.89$

$-8.29 \pm 1.54, -9.38 \pm 1.54, -1.13 \pm 1.09$

O_2 concentration 8 mg/l

$\hat{Y} = 75.54 + 3.11x_1 - 1.30x_2 - 6.96x_1^2 - 8.36x_2^2 - 1.63x_1x_2$

with standard errors of

$75.54 \pm 2.75, 3.11 \pm 1.53, -1.30 \pm 1.53$

$-6.96 \pm 2.65, -8.36 \pm 2.65, -1.63 \pm 1.87$

Holkham Bay

O_2 concentration 2 mg/l

$\hat{Y} = 69.62 + 1.06x_1 - 1.69x_2 - 9.52x_1^2 - 2.01x_2^2 - 0.70x_1 1_2$

with standard errors of

$69.62 \pm 2.26, 1.06 \pm 1.23, -1.69 \pm 1.23$

$-9.52 \pm 2.15, -2.01 \pm 2.15, -0.70 \pm 1.51$

O_2 concentration 5 mg/l

$\hat{Y} = 84.67 + 1.37x_1 - 0.37x_2 - 9.39x_1^2 - 4.78x_2^2 + 3.81x_1x_2$

with standard errors of

$84.67 \pm 2.97, 1.37 \pm 1.63, -0.37 \pm 1.63$

$-9.39 \pm 2.81, -4.78 \pm 2.81 \quad 3.81 \pm 1.99$

O_2 concentration 8 mg/l

$\hat{Y} = 84.30 + 2.53x_1 - 0.64x_2 - 9.43x_1^1 - 2.95x_2^2 + 2.26x_1x_2$

with standard errors of

$84.30 \pm 4.49, 2.53 \pm 2.46, -0.64 \pm 2.46$

$-9.43 \pm 4.26, -2.95 \pm 4.26, \quad 2.26 \pm 3.07$

The anovar table may be found in Appendix 8.

The fitted response surfaces derived from the equations by regression analysis are illustrated in diagram 18. The most interesting feature of these surfaces relates to the location of their centres. This will be examined below, but first the other aspects of the surfaces will be discussed.

At an oxygen concentration of 2 mg/l O_2 the lengths of the axes for x_1 and x_2 are generally similar for the same response level in both Bay and Salts Hole samples. The areas encompassed by the 85% survival contours for example, are similar. The contours are broad and confirm that G. duebeni is a euryplastic species with higher salinity instrumental as the strongest limiting factor. This is partic-

Table 12

Gammarus duebeni. Percentage survival of adults for immersion of 120 hours in 9 salinity temperature combinations at 3 oxygen concentrations

Salts Hole Samples:

Trial	O_2 conc mg/l	5°C Salinity 10	20	30 ‰	10°C Salinity 10	20	30 ‰	15°C Salinity 10	20	30 ‰
1	2	76	76	74	92	88	82	72	72	72
2		72	80	84	98	94	82	72	80	84
mean		74	78	79	90	91	82	72	76	78
1	5	92	84	84	88	100	92	88	92	76
2		84	92	86	96	100	86	92	92	84
mean		88	88	85	92	100	89	90	92	80
1	8	76	80	94	76	100	94	72	72	80
2		68	80	92	84	96	80	70	84	80
mean		72	80	93	80	98	82	71	78	80

Holkham Bay Samples

1	2	70	72	64	92	88	88	76	78	72
2		76	80	72	82	88	80	80	72	72
mean		73	78	80	87	88	84	78	75	72
1	5	98	92	84	92	98	96	84	90	80
2		92	96	90	96	100	100	94	96	88
mean		95	94	87	94	99	98	89	93	84
1	8	88	80	84	96	100	100	92	92	98
2		92	90	90	100	100	92	88	92	92
mean		90	85	87	98	100	96	90	92	95

ularly true of the Salts Hole specimens. The slight rotation of the axes in the Bay population suggests interaction between salinity and temperature factors where, as salinities increase, maximum resistance can be maintained only when coupled with decrease in temperature. This effect is also noticeable in the Bay and Salts Hole surfaces at 5 mg/l O_2, and the Bay surface at 8 mg/l O_2. Interaction effects are negligable in the Salts Hole sample at 8 mg/l O_2. This surface also shows a reduced plasticity which is perhaps best noted by comparing the areas encompassed by the 90% survival contours in the Bay and Salts Hole plots for 8 mg/l O_2. The reason for this is obscure. It is unreasonable to argue that higher O_2 concentrations act as a limiting factor in themselves. A tentative hypothesis based on a casual observation that the gammarids swim more actively in the higher O_2 concentration, is that this activity leads to lower O_2 levels than in the 2 and 5 mg/l containers, where the animals are quiescent.

The major differences between the response surfaces relate to tolerance changes in the samples. These are best seen as changes in the location of the response surface centre and are represented in diagram 20. There were no appreciable temperature effects noted, but it was surprising that the optimum yield should be located at salinities which were lower than the recorded salinity range.from the Salts Hole and higher than the salinities in which G. duebeni is usually found in Wells Harbour. Indeed, the response centres seem to relate best to the salinities in which they have been maintained in the laboratory for several weeks. Kinne, who has worked extensively on the acclimation potential of G. duebeni does not describe any direct influence of acclimation salinity to subsequent tolerance of salinities within the experienced environmental range. Kinne (1959, 1963b).There is a noticeable difference in the Bay sample at 2 mg/l O_2 concentration. Here the optimum yield is at a lower salinity, (15.9 ‰), which implies that tolerance to higher salinities is dependent on high O_2 availability. This is in accordance with the studies performed by Kinne (1962). Surprisingly this does not seem to apply to the Salts Hole population which maintains an optimum yield close to the value of the acclimation salinity of 20‰. The Salts Hole population then is more tolerant of high salinity even when O_2 concentration is lowered. The species is well known for the environmental extremes it tolerates,however.(Gunter 1957, Davenport 1979). Such wide ranges cannot be tolerated by members of a single population, however, suggesting the existence of genetic differences in the various populations studied. (Rygg 1972, Fenchel and Kolding, 1979).

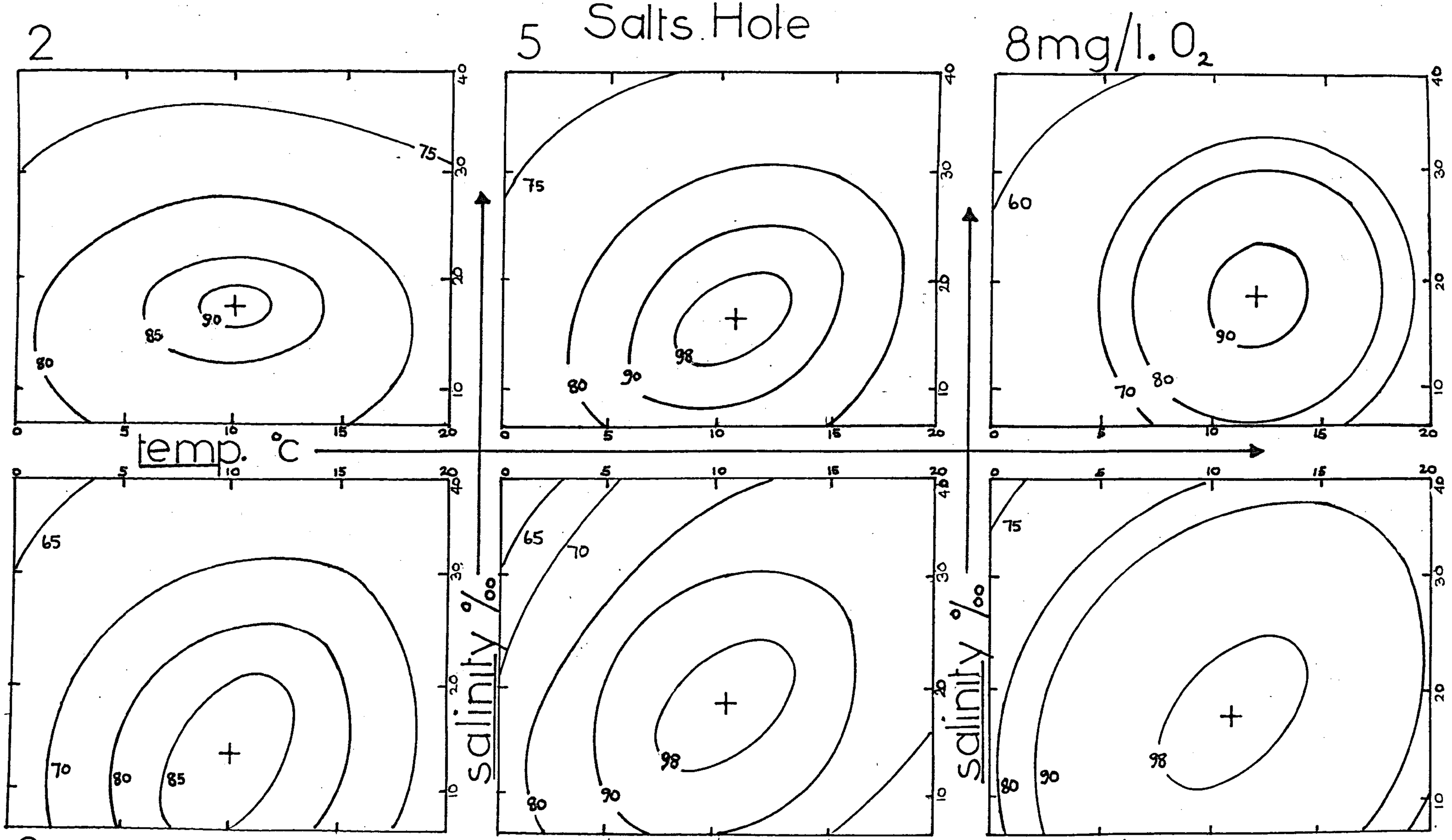

2
5 Salts. Hole
8mg/l. O_2
temp. °C
salinity ‰
2
5 Holkham Bay
8mg/l. O_2
G. duebeni, adult

FIG.18

d) The combined effects of salinity, temperature and oxygen concentration on juvenile G. duebeni.

The experimental tanks maintained at 10°C and a salinity of 20‰, and oxygenated and lit as described for the adult gammarids, (page 54) provided a large number of juveniles which were released during the summer months of 1980. No attempt to separate the animals with respect to sex or moult, was made, but samples of 50 animals taken from the Salts Hole and Bay stock tanks were tested for their response to the same temperature and salinity parameters as those used for the adults. An O_2 concentration of 2 mg/l was the only one employed in these experiments, which were performed on animals less than 4 mm in length. The experimental design was the same as that used for the adult G. duebeni.

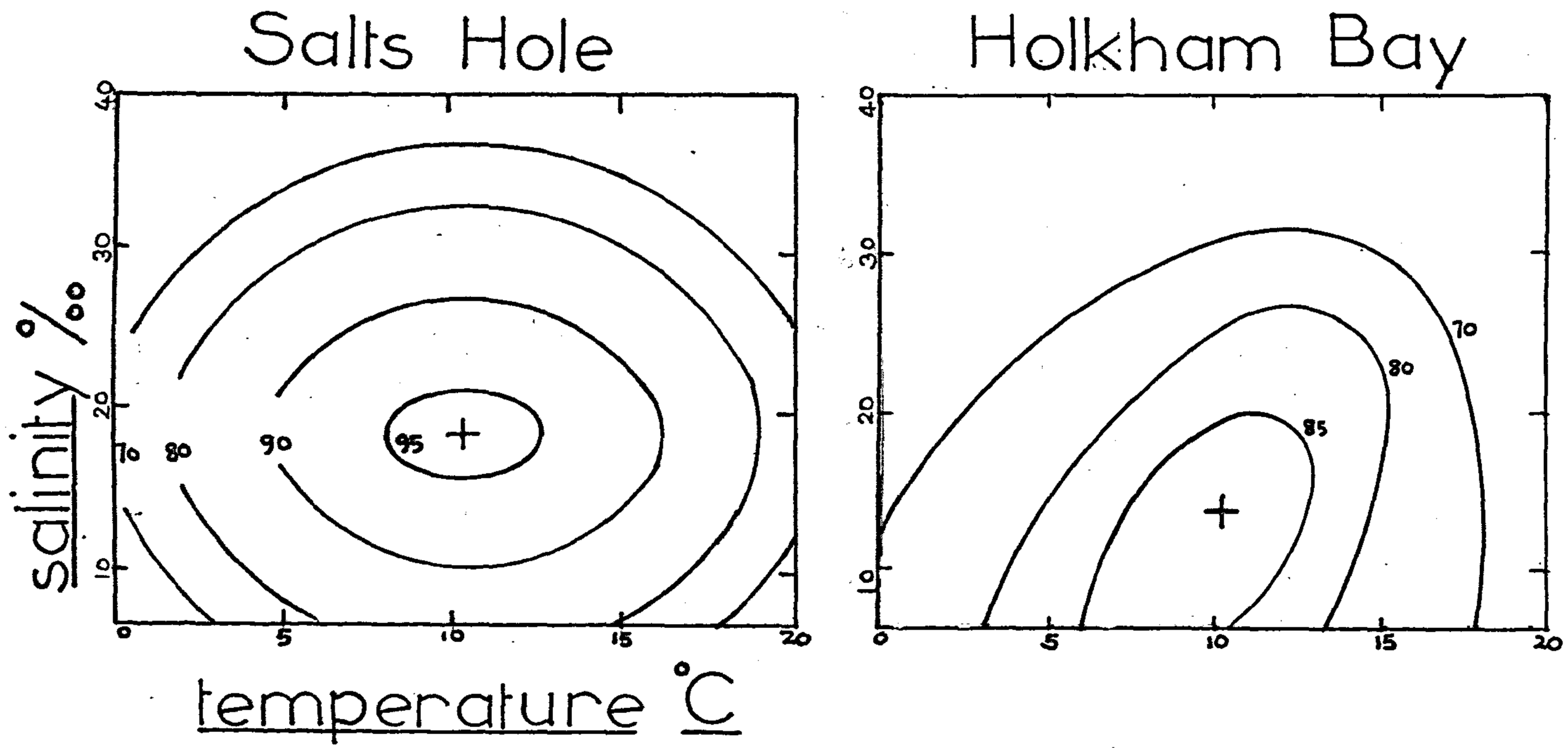
The dates of the experimental runs were 21 and 27 August, 1980. The duration of the experiment was 120 hours. Every day dead animals were removed and the O_2 concentration checked and corrected as necessary. The anovar table for the experiment may be found in Appendix 8.

Table 13 contains the replicate trial results for this experiment.

Table 13

Gammarus duebeni: Percentage survival of juveniles for immersion of 120 hours in 9 salinity - temperature combinations.

Trial	O_2 conc. mg/l	Temperature 5°C Salinity 10	20	30 ‰	10°C Salinity 10	20	30 ‰	15°C Salinity 10	20	30 ‰
Salts Hole										
1	2	80	76	68	88	100	86	90	80	78
2		74	94	76	90	92	74	74	82	74
mean		77	85	72	89	96	80	82	81	76
Holkham Bay										
1	2	76	82	76	88	82	98	72	82	88
2		84	86	92	100	94	96	92	80	88
mean		80	84	84	94	88	94	82	81	88



Gammarus duebeni, juveniles

O_2 concentration = 2mg/l.

FIG 19

The calculated equations are

Salts Hole

$Y = 77.72 + 0.43x_1 - 2.28x_2 - 9.48x_1^2 - 7.85x_2^2 - 0.37x_1x_2$

with standard errors of

77.72 ± 4.78, 0.43 ± 2.62, $- 2.28 \pm 2.62$
-9.48 ± 4.53, -7.85 ± 4.53, $- 0.37 \pm 3.20$

Holkham Bay

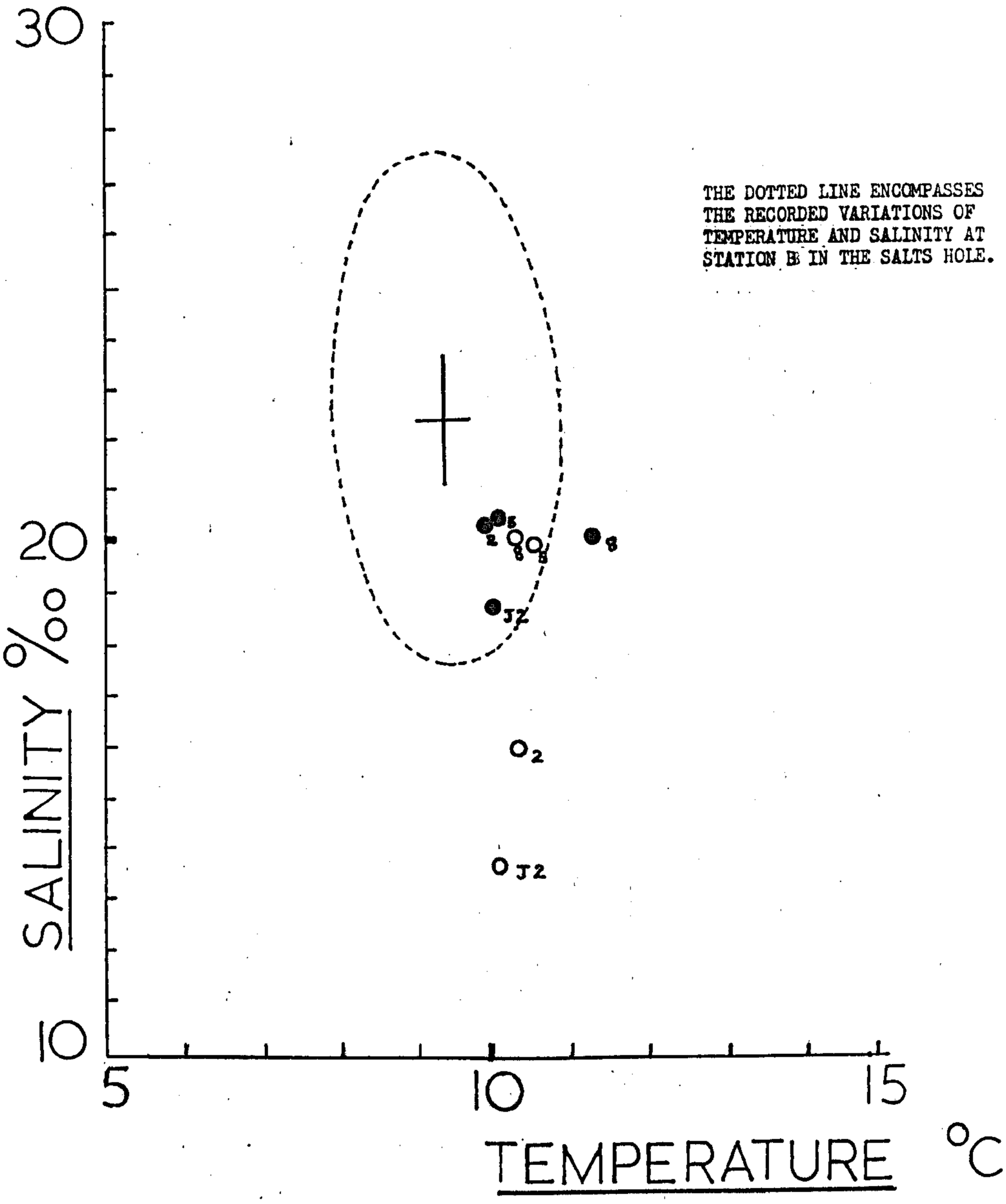
$Y = 71.42 + 0.43x_1 + 2.26x_2 - 6.43x_1^2 + 1.92x_2^2 + 0.09x_1x_2$

with standard errors of

71.42 ± 4.58, 0.43 ± 2.51, 2.26 ± 2.51
-6.43 ± 4.35, 1.92 ± 4.35, 0.09 ± 3.07

The fitted response surfaces derived from these equations by regression analysis are illustrated in diagram 19. They are very similar to the response surfaces plotted for the adults at 2 mg/l O_2 (diagram 18). In the Salts Hole samples the area encompassed by the 80% contour is closely comparable although the juveniles are able to tolerate slightly higher salinities and show increased mortalities at both higher and lower temperatures. There is no evidence of interaction between the environmental variables nor is there significant change in tolerance to them. The response centre falls within the recorded range of variability for both salinity and temperature in the Salts Hole (diagram 20). The mortalities for juveniles and adults were comparable.

In the Bay samples, correspondence between adults and juveniles was even closer. The marked interaction between temperature and salinity, absent in the pond material, is noticeable as are the increased mortalities. Both of these features support the contention that the Bay population is more susceptible to lowered O_2 concentrations, and can maintain a comparable survival only in a narrower range. In particular, the physiological mechanisms influencing survival in higher salinities seem to be especially affected. The lowered response surface centre of 13.5‰ compared to that of the adults, (15.9‰) supports this arguement.



THE RESPONSE SURFACE CENTRES OF THE SALTS HOLE SAMPLES ARE INDICATED BY ● .THOSE OF THE BAY SAMPLES BY O . THE NUMBERS 2,5,and 8 REFER TO THE OXYGEN CONCENTRATION IN mg/l. J : INDICATES JUVENILE SAMPLES, THOSE UNMARKED ARE ADULTS.

G.duebeni : RESPONSE SURFACE CENTRES

FIG.20

e) The combined effects of salinity, temperature and oxygen concentration on adult Praunus flexuosus.

Specimens of P. flexuosus were collected by wading with a push-net from the Salts Hole and from the area east of Wells Harbour, previously described (ref:928438) in March 1981. The animals were kept in aquaria as has been previously described for I. chelipes, in water of 20‰ and at 10°C. Previous experience with this species and of its high O_2 demands led to the provision of a constant oxygen supply to the tanks. Water was completely changed every 14 days. Constant illumination was maintained at 1.5×10^3 lux by striplighting. Mauchline (1971) found that in Loch Etive and the Firth of Clyde, juveniles were released principally during the early summer months and in both stock tanks juveniles were to be found during May and June. A similar experiment to that previously described for I. chelipes on page 44 was carried out to establish the salinity parameters of the response surfaces.

Fifty animals from the Salts Hole stock tank were placed in a series of 4.5 litre containers with 3 litres of SW, whose salinity ranged from 0-40‰ in 5‰ intervals. The temperature was maintained at 10 ± 0.5°C and the oxygen concentration was maintained at 5 mg/l O_2 and checked every 24 hours. Three trials were performed as before, one with all males, the second with all females and the third with a mixed population of 25 males and 25 females. Females with young in the marsupium were excluded from the trial as were any animals less than 10 mm in length. The number of animals surviving the treatment were recorded daily and any dead specimens removed.

It was decided for ease of operation to maintain the O_2 concentrations and temperatures at the same levels as those employed for I. chelipes i.e. 2, 5 & 8 mg/l O_2 and 5,10 and 15°C.

Table 14 records the results of the experiment to establish salinity parameters. The full data may be found in Appendix 7.
A Kruskal-Wallis test was performed on this data to determine whether the medians of the three replicate samples were similar. This proved to be significant at the 5% level, establishing that sex differences are not a factor in determining mortality. One hundred percent deaths were recorded at 40‰ from 96 hours and in one sample at 5‰ from 144 hours. At 120 hours, this level of mortality had not been reached by either 5‰ or 35‰ and this time was selected for the experimental duration, and the two salinities as the lower and upper parameters respectively. This established 20‰ as the middle value.

Salinity ‰	Mean % survival and standard deviation after											
	24 hours		48 hours		72 hours		96 hours		120 hours		144 hours	
5	56.67	4.11	48,00	7.48	38.00	7.12	30.00	12.75	23.33	12.84	14.67	12.26
10	64.67	5.25	58.67	4.99	56.00	4.32	54.67	4.71	50.67	7.51	49.33	9.57
15	100.00	0.00	96.67	4.71	92.67	5.74	91.33	5.01	88.67	4.11	86.67	4.99
20	96.67	2.49	96.67	2.49	92.67	2.49	90.67	3.40	87.33	5.25	87.33	5.25
25	97.33	0.94	95.33	3.77	94.67	3.40	92.67	3.40	90.67	4.11	89.33	4.99
30	86.00	5.73	76.00	4.32	66.67	6.80	61.33	4.99	59.33	6.18	56.00	5.89
35	68.67	13.18	57.33	8.22	52.00	6.35	46.67	5.73	40.00	6.53	35.33	4.11
40	41.33	15.52	18.67	8.06	5.33	4.71	0	0	0	0	0	0

Table 14 P. flexuosus % survival to a range of salinities

McLusky and Heard (1971) found that P. flexuosus would survive unfed for over 14 days and so the five day duration did not present feeding complications.

Determination of Response Surfaces: Methods

Fifty animals 11 mm or more in length were placed in 4.5 litre containers with 3 litres of SW of the appropriate salinity. Temperatures were maintained in thermostatically controlled waterbaths. Mixtures of O_2 and N_2 were bubbled into the containers for 30 minutes, the O_2 concentration checked by meter and the flasks sealed. Every day the numbers of surviving mysids were recorded, the dead specimens removed and a further aeration with the appropriate gas mixture given.

In each pair of containers one sample consisted of Salts Hole specimens, the other of Bay specimens. Pairs of containers were set up for concentrations of 2, 5 and 8 mg/l O_2 at 5, 20 and 35‰ salinity, 9 pairs of containers in total. The first experimental run was started on 2.5.81 at 5°C then on the following dates, 10°C (8.5.81) and 15°C (14.5.81). A replicate series of experiments was then repeated at 5°C (21.5.81), 10°C (27.5.81) and 15°C (3.6.81).

Determination of response surfaces: results

The percentage survival for each combination of environmental variables in given in Table 15. The equations of the response surfaces produced were of the following form;

$$\hat{Y} = b_o x_o + b_1 x_1 + b_2 x_2 + b_{11} x_1^2 + b_{22} x_2^2 + b_{12} x_1 x_2$$

The calculated equations are

Salts Hole

O_2 concentration 2 mg/l

$$\hat{Y} = 51.32 - 10.99 x_1 + 3.84 x_2 - 23.73 x_1^2 - 24.91 x_2^2 + 4.37 x_1 x_2$$

with standard errors of

51.32 ± 8.06, -10.99 ± 2.42, 3.84 ± 2.42
-23.73 ± 7.25, -24.91 ± 7.25, 4.37 ± 3.63

O_2 concentration 5 mg/l

$$\hat{Y} = 61.48 - 18.86 x_1 + 1.02 x_2 - 18.59 x_1^2 - 26.35 x_2^2 - 1.06 x_1 x_2$$

with standard errors of

$61.45 \pm 3.45, -18.86 \pm 1.89, 1.02 \pm 1.89$

$-18.59 \pm 3.27, -26.35 \pm 3.27, -1.06 \pm 2.31$

O_2 concentration 8 mg/l

$\hat{Y} = 71.76 - 18.56x_1 - 0.86x_2 - 14.06x_1^2 - 23.44x_2^2 - 0.60x_1x_2$

with standard errors of

$71.76 \pm 4.98, -18.56 \pm 2.73, -0.86 \pm 2.73$

$-14.06 \pm 4.73, -23.44 \pm 4.73, -0.60 \pm 3.34$

Holkham Bay

O_2 concentration 2 mg/l

$\hat{Y} = 44.59 - 8.20x_1 - 7.95x_2 - 17.77x_1^1 - 19.78x_2^2 + 8.80x_1x_2$

with standard errors of

$44.59 \pm 3.23, -8.20 \pm 1.77, -7.95 \pm 1.77$

$-17.77 \pm 3.07, -19.78 \pm 3.07, 8.80 \pm 2.17$

O_2 concentration 5 mg/l

$\hat{Y} = 58.70 - 18.59x_1 - 6.42x_2 - 18.26x_1^2 - 14.62x_2^2 - 2.2x_1x_2$

with standard errors of

$58.70 \pm 3.42; -18.59 \pm 1.87, -6.47 \pm 1.87$

$-18.26 \pm 3.24, -14.62 \pm 3.24, -2.20 \pm 2.29$

O_2 concentration 8 mg/l

$\hat{Y} = 68.40 - 20.37x_1 - 6.34x_2 - 15.78x_1^2 - 23.69x_2^2 - 2.48x_1x_2$

with standard errors of

$68.40 \pm 3.90, -20.37 \pm 2.14, -6.34 \pm 2.14$

$-15.78 \pm 3.71, -23.69 \pm 3.71, -2.48 \pm 2.62$

Where $\hat{Y}$ = estimated response in angular units

x_1 = temperature

x_2 = salinity

The anovar table for this experiment may be found in Appendix 8.

Table 15

Praunus flexuosus: Percentage survival of adults for immersion of 120 hours in 9 salinity - temperature combinations at three oxygen concentrations.

Trial	O_2 conc. mg/l	5°C			10°C			15°C		
		5	20	35‰	5	20	35‰	5	20	35‰
Salts Hole Specimens										
1	2	0	50	18	18	74	18	0	0	0
2		0	62	0	6	70	20	0	0	0
mean		0	56	9	12	72	19	0	0	0
1	5	26	76	28	20	80	34	0	8	0
2		36	76	48	34	90	26	0	14	0
mean		31	76	38	27	85	30	0	11	0
1	8	54	100	68	46	90	42	18	20	12
2		64	88	50	64	98	54	6	32	12
mean		62	94	59	55	94	48	12	26	12
Holkham Bay Specimens										
1	2	26	26	0	18	66	2	0	10	0
2		42	24	0	24	58	14	0	6	0
mean		34	25	0	21	62	8	0	8	0
1	5	56	70	48	56	72	32	10	16	0
2		64	60	46	54	92	28	6	8	0
mean		60	75	47	55	82	30	8	12	0
1	8	66	92	62	50	90	34	16	34	0
2		70	78	48	62	94	34	24	22	0
mean		68	85	50	56	92	34	10	28	0

The fitted response surfaces derived from these equations by regression analysis are illustrated in fig. 21 . The most interesting feature they reveal is the prominent stenoplasticity at 2 mg/l O_2 concentration. This is obvious for both the Bay and Salts Hole samples, with high mortalities recorded even at the response surface centres. Higher levels of O_2 concentration still indicate similar levels of mortality within this narrow range of variables. There is appreciable interaction between the salinity and temperature effects except for the Salts Hole 2 mg/l O_2 samples. Tolerance of both higher and lower salinities is dependent on a lower temperature. This accords well with studies on S.W. Netherlands populations of P. flexuosus by Vlasblom and Elgershuizen (1977) and is generally found in crustaceans from low salinity regimes in temperate regions, (Dorgelo 1976). McLusky (1979) has shown that this is principally a seasonal response in P. flexuosus. Survival at low salinities is enhanced in winter collected animals.

The Bay samples respond to lowered O_2 concentration by reduced tolerance to high salinities or lower temperatures. This is clearly observable in the location of the response surface centres (fig. 23). The Salt Hole samples do not show this reduced tolerance to higher salinities although the temperature effect is present. However, the only major change in location of response surface centre occurs at the lowest O_2 concentrations. The response surface centres of the Salts Hole samples are relatively close to the intersection of mean salinity and temperature levels recorded in the Salts Hole, and even the least related centre (8 mg/l O_2) is more closely located to it than any of the Bay samples.

f) Combined effects of salinity, temperature and oxygen concentration in juvenile Praunus flexuosus.

Large numbers of juveniles were released by the females in both stock tanks during April and May, with rather fewer released in June. Some of these were lost during the fortnightly water changes, but others increased by 3 up to 5 mm in length during the summer months. In an attempt to exclude older juveniles, the maximum length limit for specimens was set at 6 mm. Fifty animals were included in each sample and their response to the same salinity and temperature parameters as those employed for the adult P. flexuosus were used. The methods employed were the same as those previously described.

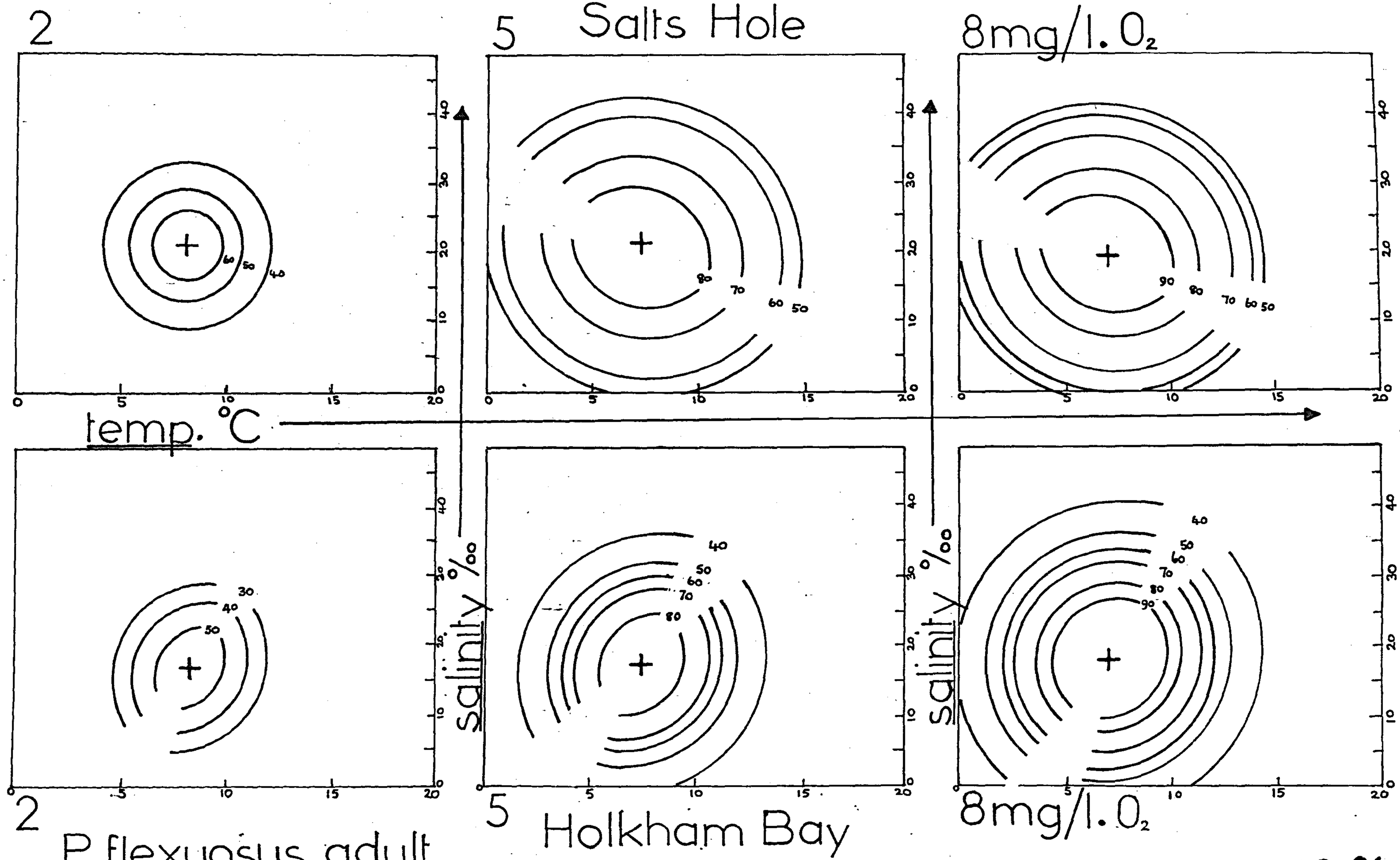

2
5 Salts Hole
8mg/l. O2
temp. °C
salinity ‰
2
5 Holkham Bay
8mg/l. O2
P. flexuosus, adult

FIG. 21

Low levels of O_2 concentration produced the most noticeable shifts in response surface centres and for this reason only 2 mg/l O_2 concentrations was examined in this experiment. The experiment was allowed to run for 120 hours starting on 15.6.81. A replicate run was carried out from 21.6.81. Dead animals were removed daily, the O_2 concentration checked and corrected as necessary. A constant surface illumination of 1.5×10^3 lux was maintained throughout the experiment. The mean values of the replicate trials are given in Table 16. The anovar table may be found in Appendix 8.

Table 16

Praunus flexuosus: Percentage survival of juveniles for immersion of 120 hours in 9 salinity - temperature combinations.

Salts Hole Specimens

Trial	O_2 conc. mg/l	5°C			10°C			15°C		
		5‰	20‰	35‰	5‰	20‰	35‰	5‰	20‰	35‰
1	2	0	68	4	42	80	17	0	0	0
2		0	74	26	18	90	23	0	8	0
mean		0	71	15	30	85	20	0	4	0

Holkham Bay Specimens

1	2	58	42	0	37	75	7	14	24	0
2		40	30	12	33	79	29	6	24	0
mean		49	36	6	35	77	18	10	24	0

The equations for the response surfaces produced were

Salts Hole

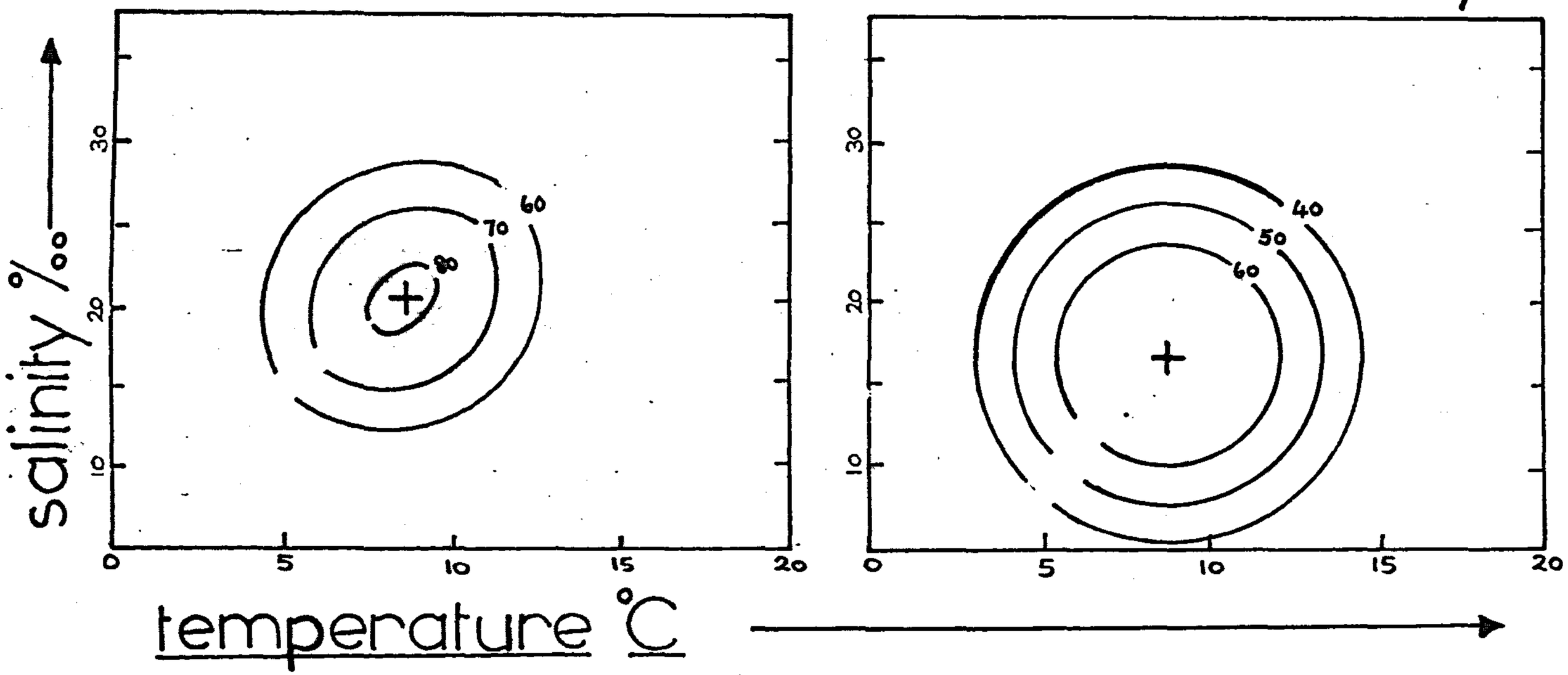
$$\hat{Y} = 62.89 - 11.72\,x_1 + 2.48x_2 - 27.79x_1^2 - 31.01x_2^2 + 5.28x_1x_2$$

with standard errors of

$$62.89 \pm 5.47,\ -11.72 \pm 2.99,\ 2.48 \pm 2.99$$
$$-27.79 \pm 5.19,\ -31.01 \pm 5.19,\ 5.28 \pm 3.67$$

Holkham Bay

$$\hat{Y} = 54.10 - 7.32x_1 - 10.78x_2 - 17.40x_1^2 - 20.36x_2^2 + 4.05x_1x_2$$



Praunus flexuosus, juveniles

O_2 concentration = 2mg/l.

FIG. 22

with standard errors of

54.10 ± 5.39, -7.32 ± 2.95, -10.78 ± 2.95

-17.40 ± 5.12, -20.36 ± 5.12, 4.05 ± 3.62

Where $\hat{Y}$ = estimated optimum yield

x_1 = temperature

x_2 = salinity

The fitted response surfaces derived from these equations by regression analysis are illustrated in fig 22. There are some noticeable differences between these samples and those of adult P. flexuosus at 2 mg/l. Both juvenile samples show an increase in capacitance demonstrated by the increased optimum yields recorded at the response surface. In the Salts Hole sample this is an increase in survival by over 20%. This may be related to the relatively lower O_2 demands of the juveniles. Since it was possible that the adults were depleting the oxygen levels appreciably, a trial was carried out over several days in which the O_2 concentration was monitored every 24 hours. Two 4.5 litre containers with 3 litres of 20‰ SW were maintained at 10°C and 50 Salts Hole juveniles were placed in one container and 50 adults in the other. Dead animals were removed daily, O_2 concentration monitored and the tank reflushed with N_2/O_2 gas mixture for 30 minutes every day in the container with the adult population. The juvenile population was monitored but not reflushed. Meter readings were checked against a micro Winkler titration. The results are recorded in table 17.

Table 17

Praunus flexuosus: Oxygen utilization mg/l and % survival in juveniles and adults.

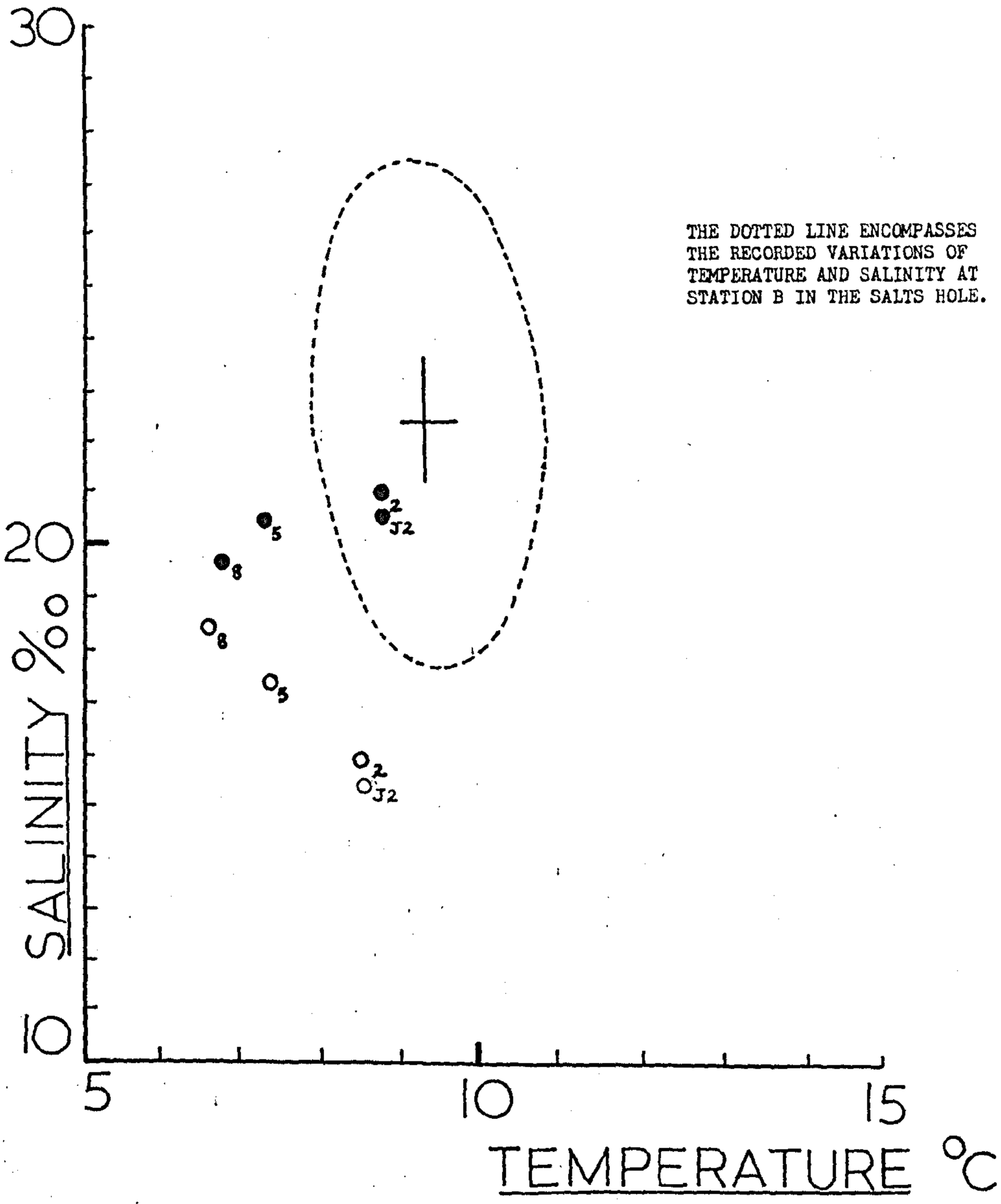
	After 24 hours	After 48 hours	After 72 hours	
Adults				
Start	2.00	2.00	2.00	meter reading mg/l
	2.10	1.96	1.94	titration mg/l
Finish	1.47	1.54	1.42	meter reading mg/l
	1.52	1.54	1.49	titration mg/l
survival	80	74	68	%

Table 17 continued

	After 24 hours	After 48 hours	After 72 hours	
Juveniles				
Start	2.00	1.70	1.58	meter reading mg/l
	1.98	1.63	1.52	titration mg/l
Finish	1.70	1.58	1.47	meter reading mg/l
	1.63	1.52	1.48	titration mg/l
Survival	92	88	80	%

Adult consumption of O_2 was higher per day than that of juveniles, but even when reflushing was not carried out in the juvenile sample and the O_2 concentration fell to levels below that recorded for the adults, there were still appreciably more juveniles surviving. The difference seems to be related more to the mechanisms of O_2 uptake, rather than the depletion of O_2 in the water.

The response surface centres (diagram 23) for juveniles correspond closely to those of the adults at 2 mg/l O_2.



THE DOTTED LINE ENCOMPASSES THE RECORDED VARIATIONS OF TEMPERATURE AND SALINITY AT STATION B IN THE SALTS HOLE.

THE RESPONSE SURFACE CENTRES OF THE SALTS HOLE SAMPLES ARE INDICATED BY ● .THOSE OF THE BAY SAMPLES BY O . THE NUMBERS 2,5 and 8 REFER TO THE OXYGEN CONCENTRATION IN mg/l. J INDICATES JUVENILE SAMPLES,THOSE UNMARKED ARE ADULTS.

P. flexuosus : RESPONSE SURFACE CENTRES

FIG.23

Electrophoretic studies using Salts Hole species.

Genetic divergence between populations of Idotea chelipes, Gammarus duebeni and Praunus flexuosus.

The use of isozymes as genetic markers has increased dramatically over the last 20 years. Isozymes offer a number of important advantages over more conventional morphological markers. Isozyme variants frequently occur spontaneously and seldom produce obviously deleterious effects. Variant alleles are generally codominant making it possible to easily and positively identify heterozygotes as well as homozygotes, and to monitor conveniently the time of expression of the parental alleles in the heterozygote. Since isozymes represent specific gene products, variants are more likely to represent specific gene lesions than are complex morphological markers. Isozymes have been used by geneticists to estimate the degree of genetic polymorphism present in populations, genetic differences within and between species, differential gene expression during development and the evolution of genes and organisms. The present study was designed to test genetic divergence at the intraspecific level of the three crustacean species by examining populations from the Salts Hole and from several stations in North Norfolk (fig.24). A number of isozyme systems were screened initially and from these two were selected for further study, leucine aminopeptidase (Lap) and malate dehydrogenase (Mdh). It is customary to employ horizontal or vertical starch gel electrophoresis to separate isozymes, but, it was found satisfactory for this study to use horizontal cellulose acetate gels which permit much more rapid analysis of the enzyme fractions.

Materials and methods: (a) collection and preparation of samples

For convenience of collecting and for general interspecific comparisons stations where all three crustaceans could be collected were chosen. This would have been relatively easily accomplished for two of the species but proved more difficult for G. duebeni which has a patchy distribution on the N. Norfolk coast, and often has to be carefully separated from the other species of Gammarus which are more fre-

quently collected. The stations chosen were:

Station 1. Brancaster Staithe (map ref. TL 793445)

This is a bed of dense clay and peat on the south side of Brancaster Harbour channel.

Station 2. Norton Creek (map ref. TL 837455)

Collections were made mainly on the south bank of the creek, which has mixed sandy and muddy substrates.

Station 3. Wells Harbour (map ref. TL 928438)

Collections were made about 0.5 km E. of the harbour where silting allows access to the channel.

Station 4 & 5. Salts Hole (map ref. TL 885451)

Two samples were collected from the Salts Hole in order to test the degree of random sampling error and so that this could be taken into account in assessing the degree of divergence in the other populations.

Station 6. Blakeney Point (map ref. TG 030455)

Collections were made about 1 km W. from the sea lane to Cley. Here the salt marshes slope down to the channel of the River Glaven.

Fig. 24 is a map showing the location of these sites.

Animals were transported live back to the laboratory. They were then frozen and maintained at -40°C until used for electrophoresis. Freezing did not seem to affect the mobility or functioning of the enzymes assayed. One hundred samples of each species from each station were collected.

Whole body samples were employed by homogenizing animals in equal volumes of distilled water and toluene, whilst the tube was immersed in an ice bath. The homogenate was centrifuged at 17,000 rpm for 20 minutes in a refrigerated centrifuge. The aqueous layer

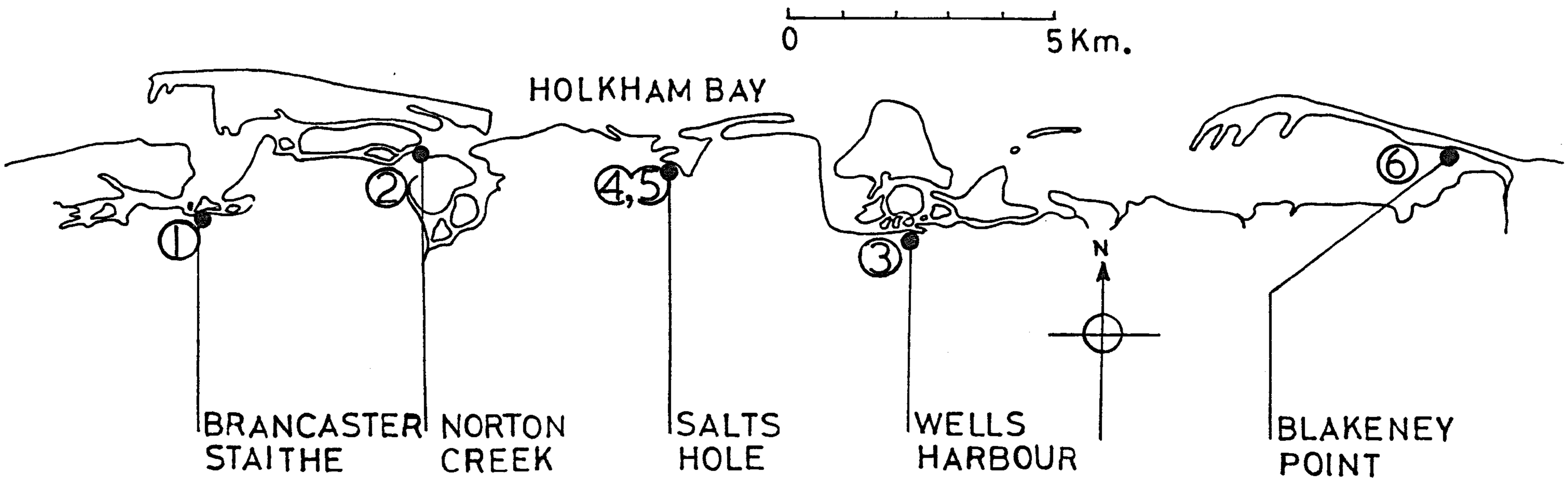


FIG. 24

SAMPLING STATIONS

was removed and stored at -40°C until used.

b) Electrophoresis of samples

Cellogel RS was used as the supporting medium. This material is described by Del Campo (1968). This cellulose acetate gel has a very uniform fine-pore structure which supports 92% of the electrolytic buffer. These small pores (0.1 to 0.5 µm) substantially reduce all band diffusion and also allow large volume samples to be applied repeatedly without spreading. Nine samples were applied to each 16 cm plate. Three 1.5 µl applications were employed for each sample. The full details of application are to be found in Appendix 9. Electrophoresis was carried out in a Cellogel tank ET.2. (Horwell; G.B.) for 50 minutes at 200 volts. The current did not exceed 12 m amps per strip. The buffer used was VTter buffer (tris-barbital buffer 0.036M) at pH 8.9.

c) Staining of gels

Two enzymes were tested for;Malate dehydrogenase (Mdh) and Leucine aminopeptidase (Lap). Sodium - L - Malate is used as the substrate for the former and L - leucyl - B - naphthyl - amide for Lap. The stains used were slight modifications of those described by Shaw and Prasad (1970) and the details of their preparation are included in Appendix 9.

Plates being tested for Mdh activity were incubated at room temperature for one hour in the stain when dark bands could be clearly seen. For Lap activity incubation was carried out at 37°C for 40 minutes. Again, dark bands were clearly visible by then. The plates were washed in several changes of 5% acetic acid.

Results

a) Malate dehydrogenase.

There were up to three distinct bands of Mdh activity recorded from these plates. Previous workers have recorded similar activity from a variety of animals (Whitt 1970, Clayton 1971). The least mobile of

these bands relates to an enzyme which is thought to be mitochondrial in origin (Mdh - 1) and is quite distinct in structure and preferred substrate to that of the supernatant enzymes (Mdh - 2 and Mdh - 3). This was established by Mann and Vestling (1968), who went on to describe the organisation of sub-unit structure within the enzymes. Mdh - 1 is a dimer which is formed from subunits produced at a single gene locus. Animals homozygous for this locus will produce only a single band in the Mdh-1 zone (eg Mdh-1^a/Mdh-1^a or Mdh-1^b/Mdh-1^b). Heterozygotes will show three bands of activity, (eg Mdh-1^a/Mdh-1^a, Mdh-1^a/Mdh-1^b and Mdh-1^b/Mdh-1^b) all three dimers having different mobilities.

The supernatant enzymes Mdh-2 and Mdh-3 are thought to be tetramers composed of subunits formed at two distinct gene loci. There are theoretically five possible combinations of subunits, (A_4, A_3B_1, A_2B_2, A_1B_3,B_4). In practice however, one of these fractions, A_3B_1, does not seem to form spontaneously, which means that in homozygotes four bands are usually locatable for Mdh-2 and for Mdh-3. In animals heterozygous for either of these loci, six bands are found. Presumably a number of the tetramers do not form spontaneously or have similar mobilities to other tetramers.

An example of this banding may be found in Plate 2, which is drawn from samples taken from P. flexuosus, where three zones of activity may be recorded, corresponding to the enzymes Mdh-1, Mdh-2 and Mdh-3. A similar pattern was observed in I. chelipes, but in G. duebeni there was no recordable evidence of Mdh-3 activity. (Plates A_1 and A_2 in Appendix 9.)

Table 18 records the frequencies of the alleles present for the Mdh allozymes in P. flexuosus.

The most interesting observations which were recorded relate to the lower frequency of heterozygosity recorded in the Salts Hole samples (4 and 5), compared to the others. Although only one locus is monomorphic, five of the recorded alleles are absent. Of particular interest here is Mdh-1^c since it is found at all the remaining stations with frequencies up to 0.26. The locus for which the Salt Hole samples are monomorphic (Mdh-2) is a characteristic shared with the Wells Harbour samples. The Blakeney Point sample (6) has as allele here (Mdh-2^d) which was not recorded at any other station. This, together with another infrequently recorded allele (Mdh-3^c) gives it the highest number of recorded alleles from these samples.

Table 18

Praunus flexuosus: allelic frequencies for Mdh enzymes
n = 100 for each station.

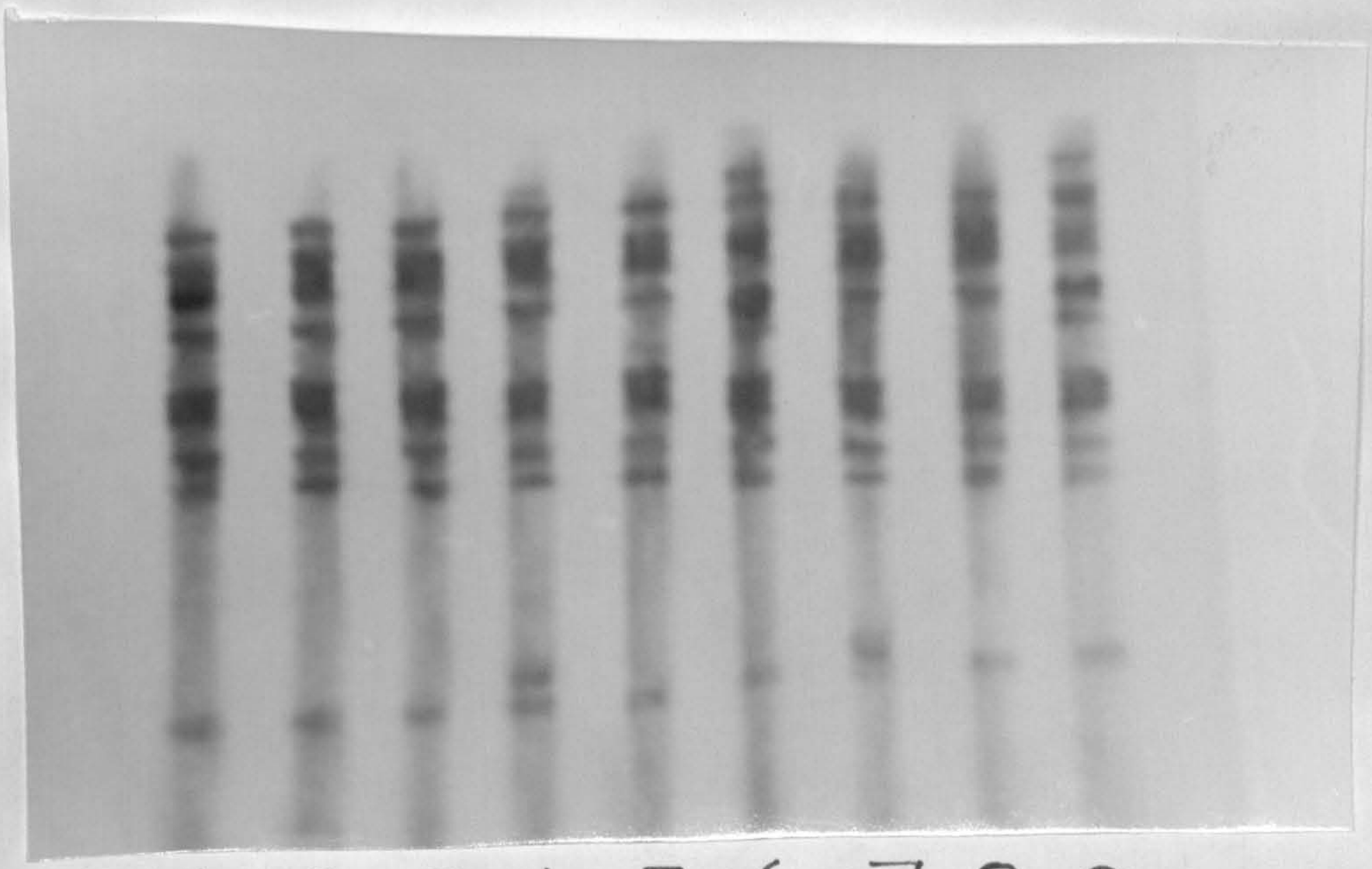
Enzyme	Allele	Stations					
		1	2	3	4	5	6
Mdh-1	-1^a	.10	.04	.02	.09	.10	.10
	-1^b	.68	.72	.83	.91	.90	.64
	-1^c	.22	.24	.15	-	-	.26
Mdh-2	-2^a	.20	.10	-	-	-	.09
	-2^b	.08	.13	-	-	-	-
	-2^c	.72	.87	1.00	1.00	1.00	.82
	-2^d	-	-	-	-	-	.09
Mdh-3	-3^a	.10	.13	.30	.68	.64	.20
	-3^b	-	-	.05	.02	.03	.09
	-3^c	-	.01	-	-	-	.02
	-3^d	.90	.86	.65	.30	.33	.69
Total	11	8	9	7	6	6	9

The distribution of alleles for Mdh in I. chelipes (see Table 19) is quite similar to those recorded above. Again the numbers of alleles present in the Salts Hole is lower than at other stations except for Brancaster Staithe. Mdh-2a, which has frequencies up to .32 at stations 1 and 6 is absent at the Salts Hole and Wells Harbour. Of more interest however, is the distribution of Mdh-3d, where frequencies of 0.96 are recorded from the Salts Hole, but where the next highest frequency Brancaster and Wells Harbour is only 0.46.

In G. duebeni (Table 20) the most obvious difference is the absence of Mdh-3, which is present in the other species studied.

2a. Expression of Mdh isoenzyme patterns in Praunus flexuosus at station 4. - the Salts Hole.

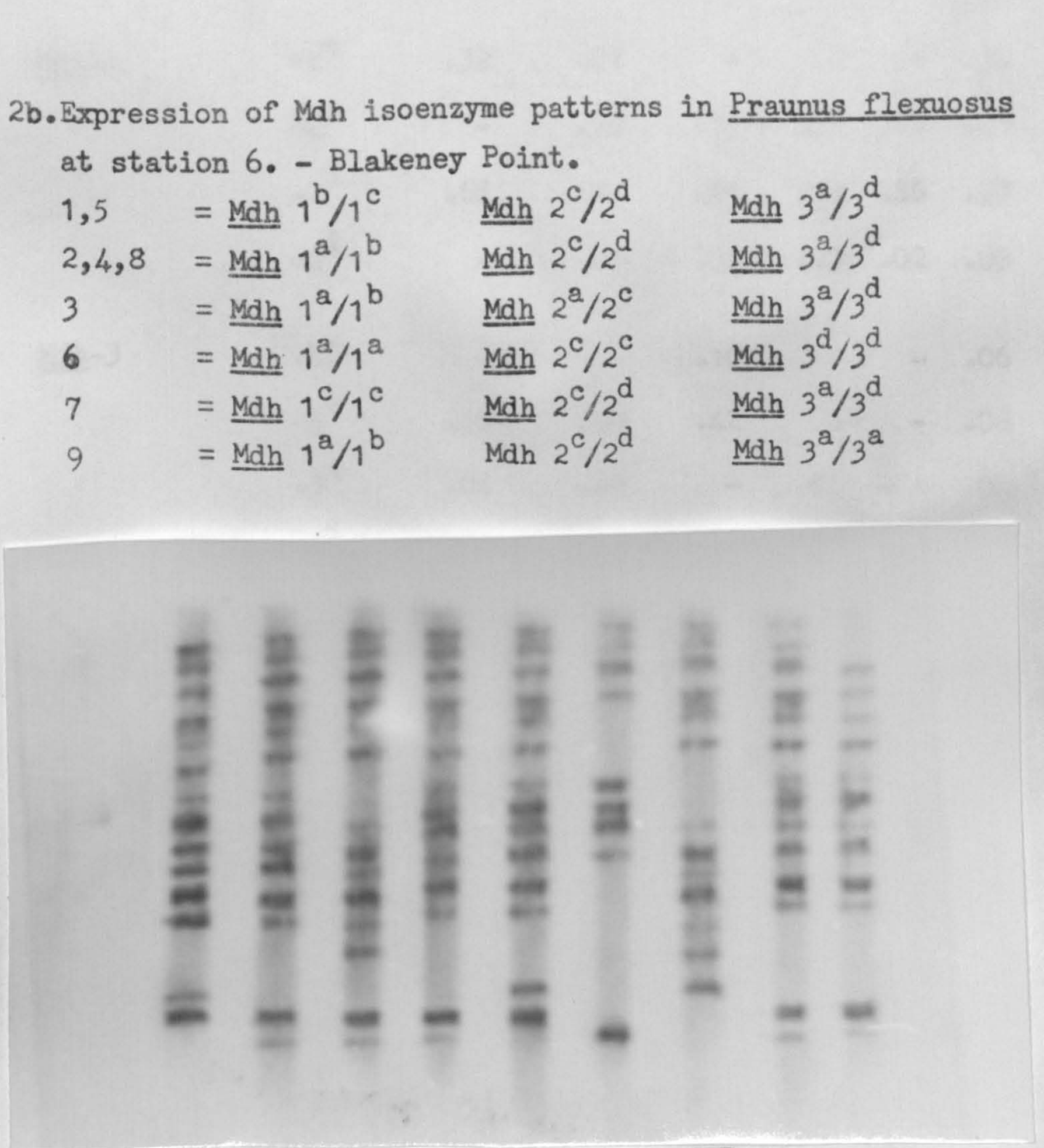
1,2,3,5,8	= Mdh $1^a/1^a$	Mdh $2^c/2^c$	Mdh $3^a/3^a$
4,7	= Mdh $1^a/1^b$	Mdh $2^c/2^c$	Mdh $3^a/3^a$
6,9	= Mdh $1^a/1^a$	Mdh $2^c/2^c$	Mdh $3^a/3^d$



1 2 3 4 5 6 7 8 9

2b. Expression of Mdh isoenzyme patterns in Praunus flexuosus at station 6. - Blakeney Point.

1,5	= Mdh $1^b/1^c$	Mdh $2^c/2^d$	Mdh $3^a/3^d$
2,4,8	= Mdh $1^a/1^b$	Mdh $2^c/2^d$	Mdh $3^a/3^d$
3	= Mdh $1^a/1^b$	Mdh $2^a/2^c$	Mdh $3^a/3^d$
6	= Mdh $1^a/1^a$	Mdh $2^c/2^c$	Mdh $3^d/3^d$
7	= Mdh $1^c/1^c$	Mdh $2^c/2^d$	Mdh $3^a/3^d$
9	= Mdh $1^a/1^b$	Mdh $2^c/2^d$	Mdh $3^a/3^a$



1 2 3 4 5 6 7 8 9

Table 19

Idotea chelipes: allelic frequencies for Mdh enzymes
n = 100 for each station.

Enzyme	Allele	Stations					
		1	2	3	4	5	6
Mdh-1	-1^a	-	-	-	-	-	.02
	-1^b	-	.07	.04	.12	.07	.10
	-1^c	.97	.91	.91	.86	.83	.84
	-1^d	.03	.02	.04	.04	.10	.02
Mdh-2	-2^a	.32	.02	-	-	-	.30
	-2^b	-	.10	.06	.07	.12	.09
	-2^c	.68	.82	.91	.90	.86	.58
	-2^d	-	.06	.03	.03	.02	.03
Mdh-3	-3^a	-	-	.10	-	-	.06
	-3^b	.50	.59	.42	-	-	.06
	-3^c	.04	.01	-	.04	.04	.04
	-3^d	.46	.28	.46	.96	.96	.32
	-3^e	-	.12	.02	-	-	-
Total	13	7	11	10	8	8	12

Table 20.

Gammarus duebeni: allelic frequencies for Mdh enzymes
n = 100 for each station.

Enzyme	Allele	Stations					
		1	2	3	4	5	6
Mdh-1	1^a	.22	.19	.20	.24	.25	-
	1^b	-	.01	.04	.09	.06	.07
	1^c	.74	.80	.72	.67	.65	.93
	1^d	.04	-	.04	-	.04	-
Mdh-2	2^a	.02	.02	-	.03	.04	.03
	2^b	.52	.37	.52	.13	.10	.29
	2^c	.39	.61	.40	.74	.78	.63
	2^d	.07	-	.08	.10	.08	.05
Total		8	7	6	7	7	7	6

There are no features which distinguish the Salts Hole samples from any of the others.

b) Leucine aminopeptidase

There were three distinct bands of Lap activity recorded in G. duebeni, but only two in I. chelipes and P. flexuosus. Similar activity has been recorded from a variety of species (Murdoch, Ferguson and Seed 1975, Wall, 1968). Kretschmer and Hanson, 1968 showed that the structure of this enzyme is complex with as many as ten subunits combining to complete its quaternary structure. Nevertheless Lap behaves as a monomer electrophoretically so that where an organism is homozygous for a given Lap, only a single band will be found in that zone. Heterozygotes produce only two bands (eg Lap 1^a/Lap 1^a, Lap 1^b/Lap 1^b). The dimer (Lap 1^a/Lap 1^b) is produced in such small amounts that the zone of activity is absent or very faint. This is true of Lap 2 and Lap 3 also.

An example of the banding patterns for Lap may be found in Plate 3 which shows the three zones associated with Lap 1, Lap 2 and Lap 3 in G. duebeni. Banding patterns for I. chelipes and P. flexuosus may be found on plates B_1 & B_2 in Appendix 9.

The distribution of allelic frequencies for P. flexuosus may be found in Table 21 below.

Table 21

Praunus flexuosus: allelic frequencies for Lap enzymes
n = 100 for each sample

Enzyme	Allele	Station					
		1	2	3	4	5	6
Lap-1	-1^a	.09	-	.12	-	-	.02
	-1^b	.91	1.00	.88	1.00	1.00	.98
Lap-2	-2^a	-	.03	.12	.02	-	.13
	-2^b	.96	.95	.84	.98	1.00	.87
	-2^c	.04	.02	.04	-	-	-
Total	5	4	4	5	3	2	4

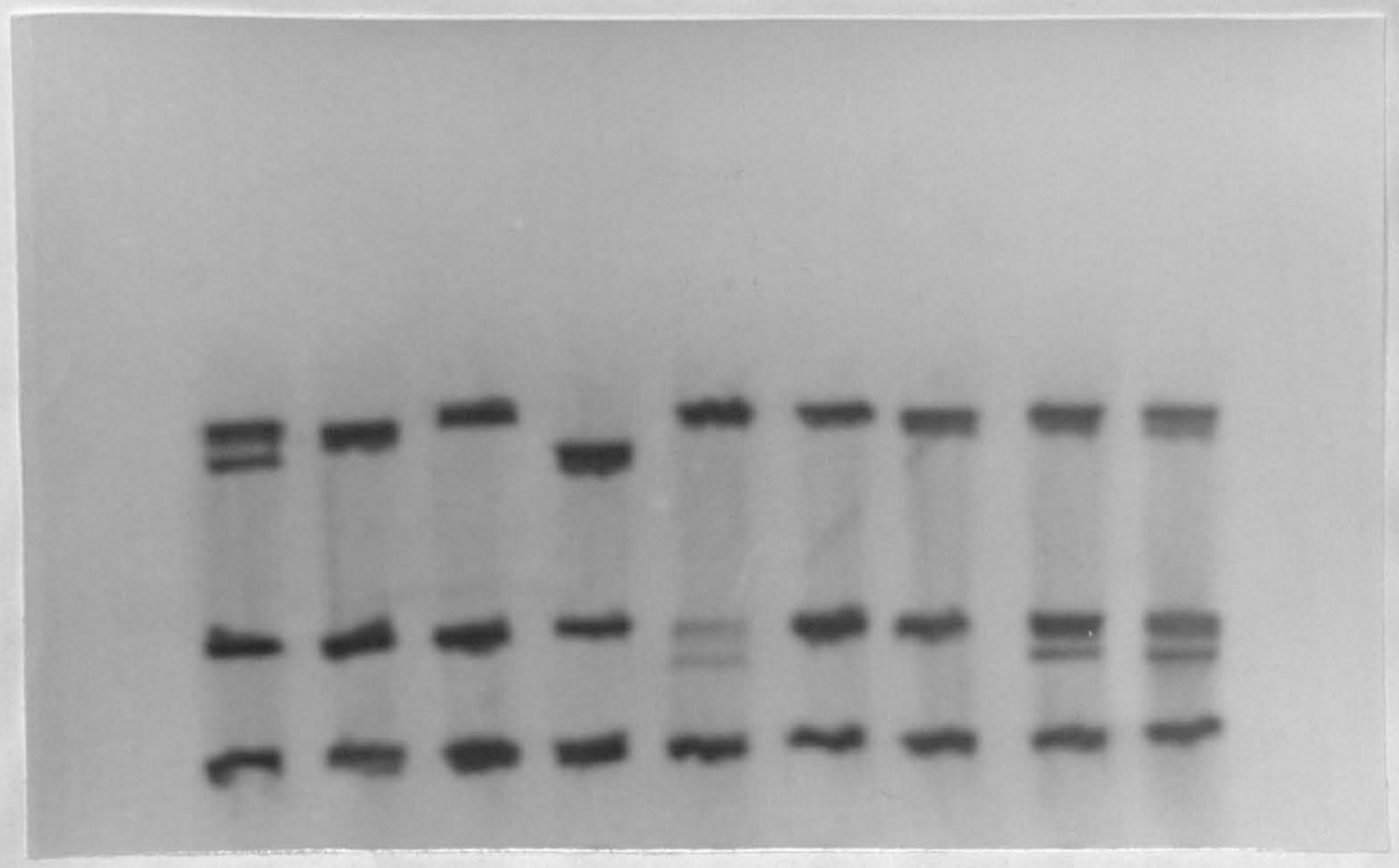
Both the Salts Hole and Norton creek populations appear to be monomorphic for Lap-1^b. Lap-1^b has high frequencies at all stations. Lap-2^c has low frequencies at stations 1-3 and is not recorded at the Salts Hole or Blakeney Point. There is a lower level of heterozygosity in the Salts Hole population than in any of the others.

For I. chelipes a broadly similar distribution of alleles is found (Table 22).

The Salts Hole and Norton Creek populations are monomorphic for both Lap enzymes. The Blakeney Point population shows the highest degree of heterozygosity, all five recorded alleles being present here.

3 a. Expression of Lap isoenzyme patterns in Gammarus duebeni at station 5. - the Salts Hole.

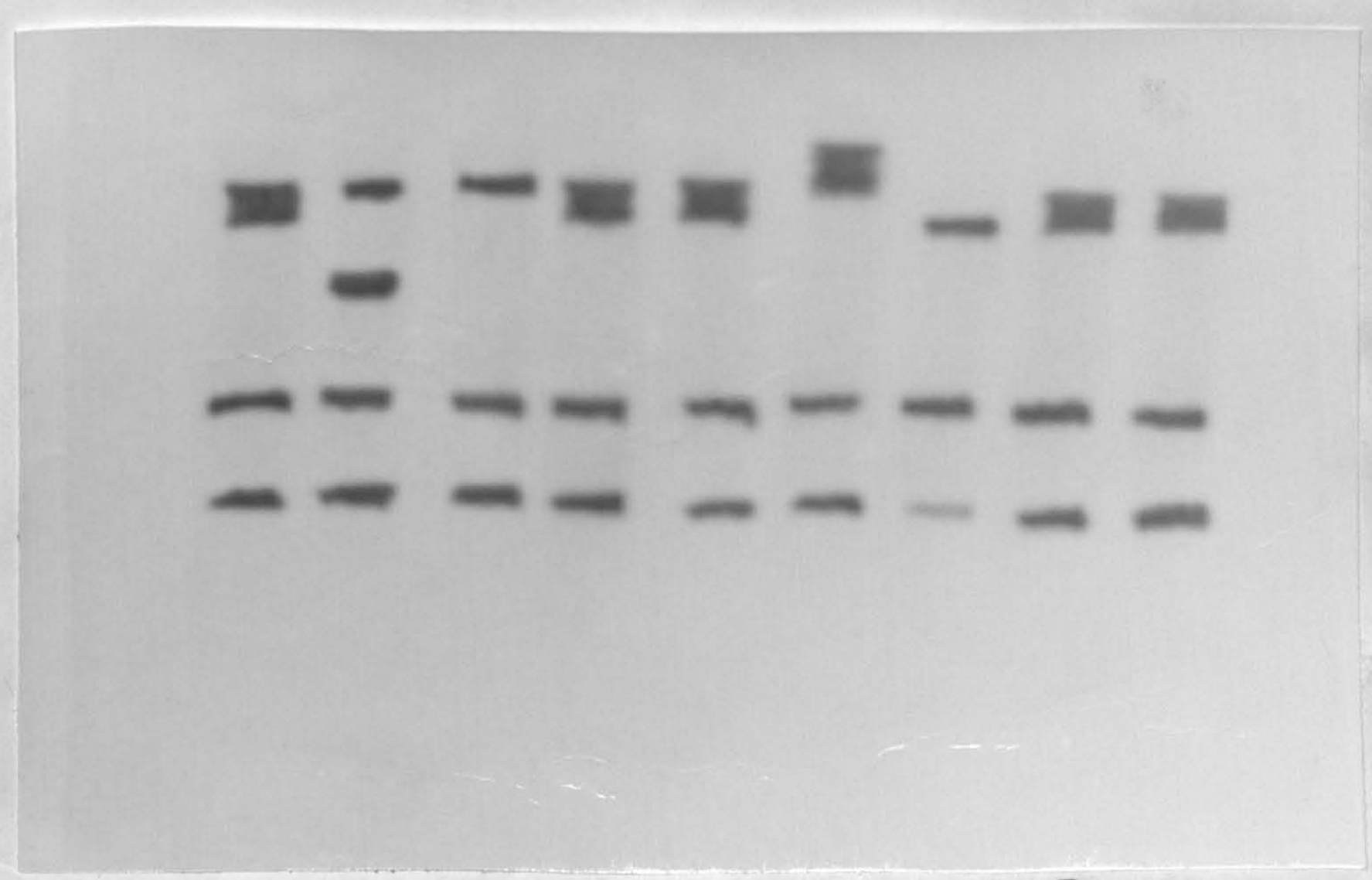
1	= Lap $1^a/1^a$	Lap $2^b/2^b$	Lap $3^b/3^c$
2,3,6,7	= Lap $1^a/1^a$	Lap $2^b/2^b$	Lap $3^c/3^c$
4	= Lap $1^a/1^a$	Lap $2^b/2^b$	Lap $3^b/3^b$
5,8,9	= Lap $1^a/1^a$	Lap $2^a/2^b$	Lap $3^c/3^c$



1 2 3 4 5 6 7 8 9

3 b. Expression of Lap isoenzyme patterns in Gammarus duebeni at station 6 - Blakeney Point.

1,4,5,8,9	= Lap $1^a/1^a$	Lap $2^b/2^b$	Lap $3^b/3^c$
2	= Lap $1^a/1^a$	Lap $2^b/2^b$	Lap $3^a/3^c$
3	= Lap $1^a/1^a$	Lap $2^b/2^b$	Lap $3^c/3^c$
6	= Lap $1^a/1^a$	Lap $2^b/2^b$	Lap $3^c/3^d$
7	= Lap $1^a/1^a$	Lap $2^b/2^b$	Lap $3^b/3^b$



1 2 3 4 5 6 7 8 9

Table 22

Idotea chelipes: allelic frequencies for Lap enzymes
n = 100 for each station

Enzyme	Allele	Stations					
		1	2	3	4	5	6
Lap-1	-1^a	-	-	-	-	-	.05
	-1^b	1.00	1.00	.96	1.00	1.00	.83
	-1^c	-	-	.04	-	-	.12
Lap-2	-2^a	.13	-	.10	-	-	.04
	-2^b	.87	1.00	.90	1.00	1.00	.96
Total	5	3	2	4	2	2	5

In G. duebeni three enzymes are locatable but for one Lap-1 all populations are monomorphic. This may be seen in Table 23.

Table 23

Gammarus duebeni: allelic frequencies for Lap enzymes
n = 100 for each station

Enzyme	Allele	Station					
		1	2	3	4	5	6
Lap-1	-1^a	1.00	1.00	1.00	1.00	1.00	1.00
Lap-2	-2^a	.04	-	-	.12	.13	-
	-2^b	.96	1.00	1.00	.88	.87	1.00
Lap-3	-3^a	-	.06	-	-	-	.08
	-3^b	.22	.22	.04	.10	.13	.20
	-3^c	.72	.68	.94	.90	.87	.68
	-3^d	.06	.04	.02	-	-	.04
Total	7	6	6	5	5	5	6

In addition to Lap-1, three stations are monomorphic for Lap-2^b. This allele has the lowest frequency at the Salts Hole. Neither Lap-3^a nor Lap-3^d were recorded in the Salts Hole population, but neither alleles reach high frequencies at any other station.

The most interesting observation which this experiment reveals is the relatively few alleles to be identified in the Salts Hole population. Only 31 of the 49 alleles located for the three species of crustaceans taken together were present. Table 24 summarises this information.

Table 24

Total number of alleles recorded at stations 1 - 6

	Species	Station 1	2	3	4	5	6	Total alleles scored
Lap enzymes	P. flexuosus	4	4	5	3	2	4	5
	G. duebeni	6	6	6	5	5	5	7
	I. chelipes	3	2	4	2	2	5	5
Mdh enzymes	P. flexuosus	8	9	7	6	6	9	11
	G. duebeni	7	6	7	7	7	6	8
	I. chelipes	7	11	10	8	8	12	13
Total		35	37	39	31	30	41	49

The observed allelic frequencies at each locus in the six populations were analysed for genetic similarities using the statistic of genetic identity developed by Nei (1972).

The probability that two alleles from different populations are the same is

$$I_j = \frac{\sum x_i y_i}{(\sum x_i^2 \sum y_i^2)^{1/2}}$$

Where X_i and y_i represent the frequencies of the i^{th} allele in populations x and y respectively. The mean genetic identity (I) over several loci is

$$I = \frac{J_{xy}}{(J_x J_y)^{1/2}}$$

Where Jx, Jy and Jxy are the arithmetic means over all loci Σx_i^2, Σy_i^2 and $\Sigma x_i^2 y_i^2$ respectively.

The pair wise genetic identities for the three species are given in Table 25. The method from which this is derived is to be found in Appendix 9. The values range from 0.887 to 0.991 (excluding the replicate samples 4 and 5 from the Salts Hole), with a mean genetic identity of 0.954 ± 0.017. The mean identity of the Salt Hole replicates is 0.998 ± 0.018. Data on genetic similarities are available for a number of organisms related to various taxonomic levels (Ayala 1975, Avise 1976).

An extensive study of the Willistoni group of Drosophila (Ayala et al, 1974) reveals the following levels of genetic divergence calculated with Nei's identity statistic for taxa of increasing evolutionary divergence.

Table 25

Genetic identity for pair-wise comparisons at six stations for populations of Gammarus duebeni, Praunus flexuosus and Idotea chelipes.

Species	Stations	1	2	3	4	5
	2	0.988				
	3	0.989	0.976			
G. duebeni	4	0.941	0.970	0.948		
	5	0.945	0.970	0.951	0.998	
	6	0.972	0.991	0.962	0.962	0.962
	2	0.989				
	3	0.966	0.979			
P. flexuosus	4	0.906	0.968	0.968		
	5	0.907	0.970	0.966	0.998	
	6	0.981	0.989	0.980	0.930	0.934
	2	0.972				
	3	0.975	0.982			
I. chelipes	4	0.917	0.903	0.947		
	5	0.917	0.900	0.944	0.999	
	6	0.981	0.974	0.963	0.898	0.894

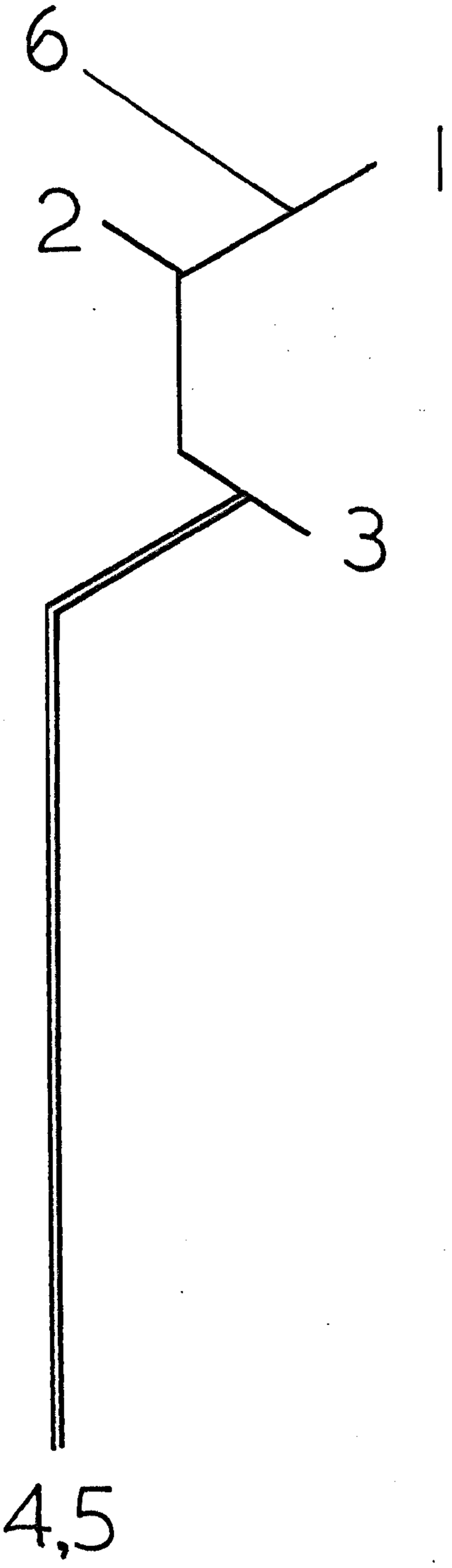
conspecific populations	I	=	0.970 ± 0.006
sub species	I	=	0.795 ± 0.013
semi-species	I	=	0.798 ± 0.026
sibling species	I	=	0.517 ± 0.024
non sibling species	I	=	0.352 ± 0.023

Studies of most other organisms have shown remarkable agreement with these figures.

Most of the pair-wise comparisons of populations in the present study fall within the normal range for conspecific populations. However, lower values occur between certain pairs. The notable ones are 0.941/0.945 between Brancaster Staithe and the Salts Hole populations for G. duebeni, 0.906/0.907 between the same stations for P. flexuosus and 0.898/0.894 between Blakeney Point and the Salts Hole populations for I. chelipes. Particularly in the last species genetic divergence between the Salts Hole and other populations is greater than between any other populations. Nevertheless the genetic identities even at their most divergent correspond more closely to a conspecific level than to a subspecific or semispecific level.

Clearly all the populations, with the probable exception of the Salts Hole at the present time, are able to hybridize since limited migration presumably takes place between them. An evolutionary tree model is not adequate for depicting relationships between conspecific populations because it does not permit hybridization or mixing of populations after an initial separation (Morton et al 1968). A minimum length spanning network, which represents relationships between groups at a single time is a more appropriate way to depict such populational relations. A network using pairwise measures of genetic distances has been developed by Thompson (1973). The programme used, called MINITREE is a modification of earlier programmes by A. Edwards and A. Cornfield. The programme seeks the minimum spanning network between n populations in n - 1 dimesional space.

MINIMUM LENGTH SPANNING NETWORK BETWEEN FIVE POPULATIONS OF Idotea chelipes.



1. Brancaster Staithe
2. Norton Creek.
3. Wells Harbour
4. } Salts Hole
5. }
6. Blakeney Point.

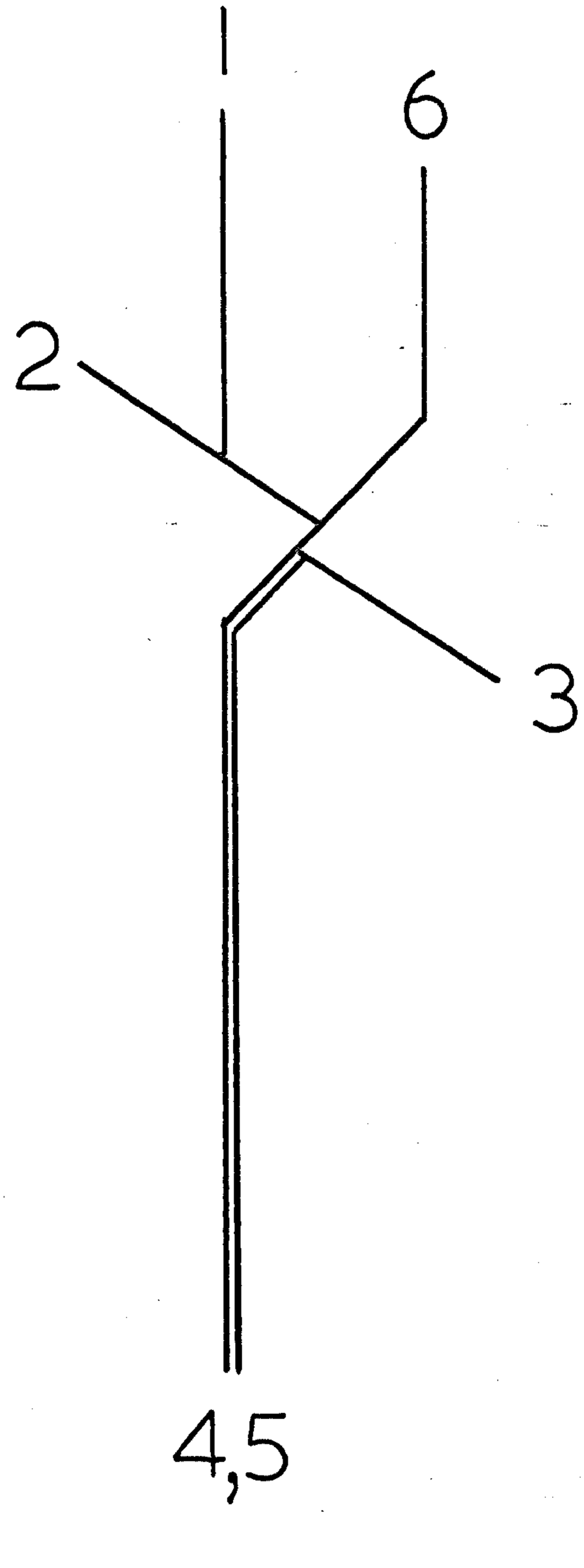
FIG. 25

The minimum length spanning network between the five populations of I.chelipes is projected in two dimensions in fig.25. Rotation is free around any of the internal nodes,thus it is not possible to tell the distance between two points separated by one or more nodes except by tracing along the lines connecting them.As can be seen from fig.25, the similarity of populations does not correlate with geographic position ,nor is it possible to attribute genetic similarity to any environmental parameter such as wave exposure, substratum or temperature.

What does emerge clearly is that the genetic divergence between the Salts Hole population and any other station is greater than between the other stations.

This characteristic is also found in populations of P.flexuosus, fig. 26, and for G.duebeni, fig 27.

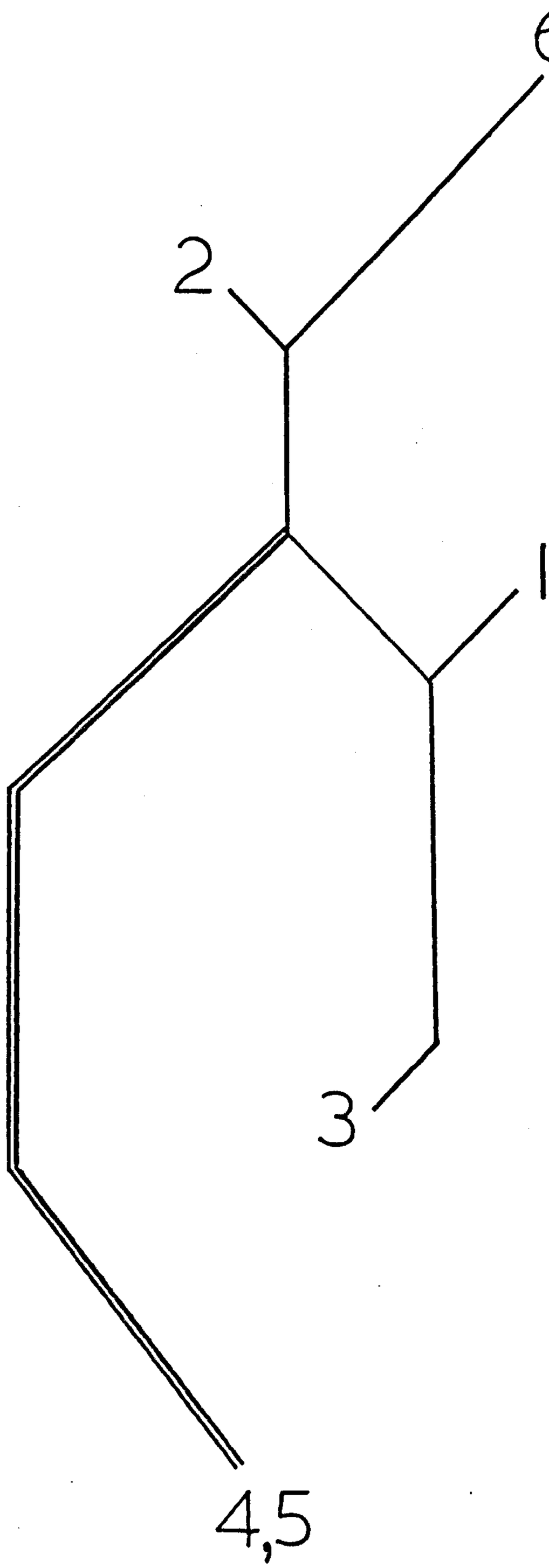
MINIMUM LENGTH SPANNING NETWORK BETWEEN FIVE POPULATIONS OF Praunus flexuosus.



1. Brancaster Staithe
2. Norton Creek.
3. Wells Harbour.
4. } Salts Hole.
5. }
6. Blakeney Point.

FIG. 26

MINIMUM LENGTH SPANNING NETWORK BETWEEN FIVE POPULATIONS OF Gammarus duebeni.



1. Brancaster Staithe.
2. Norton Creek.
3. Wells Harbour.
4. } Salts Hole
5. }
6. Blakeney Point.

FIG. 27

D I S C U S S I O N

The Salts Hole and its fauna offers to the interested scientist a unique phenomenon, a natural brackish water aquarium maintained for some 250 years with a fauna largely derived from the salt marshes which once occupied the area where the pond now lies.

All the animal species recorded in Hunt (1971) were observed in the Salts Hole and several additional species were noted. These were such that they may have been overlooked in the past, however, and do not suggest that there has been any substantial change in the fauna. Of those species present in the Salts Hole, a few are typically marine (intertidal) species, but most are more often to be found in brackish or estuarine conditions. Those species which in Britain are often or usually found in salinities less than that of sea water include the polychaetes Nereis diversicolor and Pygospio elegans, the oligochaete Clitellio arenarius, the copepods Mesochra liljeborgi and Eurytemora velox, the isopods Sphaeroma rugicauda and Idotea chelipes, the amphipods Gammarus duebeni, Microdeutopus gryllotalpa and Corophium volutator: the mysid Praunus flexuosus; the decapod Palaemonetes varians, the gastropods Hydrobia ulvae and H. neglecta and the bivalve Cerastoderma glaucum.

In comparison marine (intertidal) species are poorly represented, the sea anemone Sagartia troglodytes var. ornata; the nemertine Lineus gesserensis, the annelids Capitella capitata and Arenicola marina, the copepod Acartia clausi and the gastropod Littorina rudis.

The Salts Hole therefore supports a predominantly brackish-water fauna. The occurrence of the plants Ruppia cirrhosa and Chaetomorpha linum are also peculiar to the brackish type of habitat (den Hartog, 1970).

The studies undertaken on the Salts Hole have established that the pond possesses a remarkably stable environment for a brackish-water habitat. The tideless nature of the pond ensures that its fauna is never exposed to the extremes of air temperatures or to risks of desiccation. The water entering the pond remains within the temperature range $9.5^{\circ} \pm 1.5^{\circ}C$ and within the salinity range $25.1 \pm 3.3‰$ NaCl. pH values of the spring water were close to pH 7.4 and the water was virtually anaerobic. By the time water leaves the pond only the oxygen concentration has changed appreciably - the water now being more or less saturated.

The hydrographic survey carried out during 1975 also attested to the environmental stability. However, in one respect, an unexpected irregularity was uncovered. The pond, shallow as it is, established a halocline. In 1975, this broke down during late autumn, but observations in other years suggest that this may not always be the case. During the summer months the production of algae is reduced, presumably due to depletion of nutrients in the surface waters because the halocline prevents water mixing. The substratum of the Salts Hole is also not uniform. As stations on the northern margin of the shore indicate shingle, with sand in the interstices, predominates. This intergrades with coarse sand and then fine sand to give a few stations on the southern margin composed of silty despoits. There is also a shingle bed in the centre of the pond. North of this is a kidney-shaped depression, which includes the deepest point of the pond (2.8 m). The majority of stations are less than two metres in depth. The distribution of 3 species of macrobenthos correlated significantly with the distribution of substratum particle size, (Nereis diversicolor, Corophium volutator and Cerastoderma glaucum). Four species of macrobenthos correlated significantly with the distribution of water depth (Capitella capitata, C. volutator, Littorina rudis and Hydrobia ulvae). All these species were restricted to shallow regions.

Both the production of zooplankton and the estimated densities of selected species clearly indicate that compared to more typical estuarine conditions the Salts Hole is deficient in trophic resources. There was no accumulation of detritus and the number of detritivores is low.

The laboratory studies carried out on Gammarus duebeni, Idotea chelipes and Praunus flexuosus have established that genetic adaptation is taking place in the Salts Hole populations of all three species. The relative roles of random genetic drift and selection in bringing this about are discussed in detail later.

Finally, electrophoretic studies show that the number of alleles recorded for each species is lower in the Salts Hole than at the other stations and also that the genetic divergence between this station and any other is greater than between any other pair.

This then constitutes the core of the findings of this study, most of which have already been described in detail. Because of its unusual nature, the Salts Hole provides an ideal habitat to test hypotheses of genetic variability and species diversity. There are very few examples where a population has been isolated for a known short period

of time, and where comparisons have been drawn between this population and the populations at large. Matzke and Druger (1977) studied the evolutionary divergence of two populations of Drosophila pseudoobscura maintained under different environmental conditions for many generations (fitfeen years), which were then examined for numerous morphological and physiological traits. The two populations showed divergence with respect to some of these components but not others. .

Since these.. were entirely artificially maintained cultures, however, they could give little indication of the results that a population in the wild, with fluctuating resources and variable population numbers, would produce.

There are several hypotheses that predict relationships between genetic variability and stability of the environment. One, largely based on theoretical arguments (Levins, 1968) predicts low genetic variability in environments that are stable in physical factors such as climate and wave action. Proponents of this hypothesis have specifically predicted low levels of genetic polymorphism in tropical coral reef settings and the deep sea. Various tests of this hypothesis have produced results incompatible with these predictions (Gooch and Schopf, 1972; Ayala et al 1973). Ayala et al (1975a), found high levels of genetic variability in deep sea asteroids despite the acknowledged stability of that environment. Furthermore, intermediate or low levels of genetic polymorphism have been reported in marine invertebrates from temperate waters (Selander et al, 1970: Schopf and Murphy, 1973) and from shallow Antarctic waters (Ayala et al, 1975b).

An alternative hypothesis predicts that genetic variability should be lowest in environments that are most highly seasonal or otherwise temporally variable in trophic resources. This hypothesis was advanced to account for the observation that genetic variability in benthic marine species correlates with patterns of marine species diversity. Valentine (1971), first put forward this hypothesis, and developed it further to account for discrepancies in the paleontological record (Valentine, 1973). In ecosystems of rich species diversity, levels of genetic variability are generally high. Unfortunately, data on the seasonality of trophic resources are available for only a few scattered stations in the seas. The general pattern of trophic stability must be inferred from whatever data is available on productivity, together with hydrographic, chemical and meteorological information. In general the productivity of the oceans is most seasonal in the highest latitudes where there may be only one short but pronounced bloom

per year. Extensive studies on several species of krill in Pacific and Antarctic waters supported this hypothesis (Valentine and Ayala, 1976), where the trophic stability of tropical waters permitted higher levels of genetic polymorphism. The hypothesis is fraught with complications however. Estimation of the trophic resource regimes of animals may prove difficult, especially because the feeding of particular populations may either lessen or increase the effects of variations in primary production. Detritus feeders provide an example of this, for they may exploit over long periods of time, resources generated during short bursts of productivity. On the other hand, species which stop feeding during reproductive phases may have seasonal growth patterns even in areas of stable productivity.

Valentine expresses the hypothesis in terms of 'grainedness'. In environments which are <u>temporally coarse-grained for trophic resources</u>, that is to say, unstable, such as high latitudes or regions of highly seasonal or intermittent upwelling, species should pursue a relatively fine-grained spatial strategy, i.e. they must be flexibly adapted to tolerate a wide range of habitat conditions and to accept a wide variety of food items. Genetically, this means that those alleles with products that function in the widest range of conditions are favoured by selection. The gene pool therefore should come to consist of only the more favourable alleles and only a few flexible genotypes should be found. In environments that are <u>temporally fine-grained for trophic resources</u>, such as the tropics or deep sea, species may pursue relatively coarse-grained spatial strategies and should tend to be food or habitat specialists. This tendency must grow out of competition for limited but stable resources. Alleles which are slightly more advantageous in a certain restricted habitat condition, or which confer an advantage to heterozygotes, should be accumulated in the gene pool.

The trophic resource stability hypothesis has recently come in for some adverse criticism. Siebenaller (1978) working on the deep sea gastropod <u>Bathybembix bairdii</u> found that his results did not correlate with it, finding little diversity in his populations. Similarly, Buroker (1980), examining the hypothesis directly by studying the genetic variation of oysters from the N. pole to the equator found no variation that could be related consistently to latitude. Yet, Mulley and Latter (1980) working on australian Penaeid prawns state successful and widespread marine invertebrate species occupying markedly heterogeneous physical and trophic environments may be <u>characterized</u> by extremely low levels of genetic variation.

The argument then goes on. To what extent can studies of the Salts Hole fauna support or refute either of these hypotheses?

First it was necessary to establish whether the pond presented an environment which could be defined as stable or at least more stable than the surrounding salt marshes. It became clear that the conditions within the pond showed remarkably little short-term fluctuation in salinity, temperature and oxygen concentration. There was a seasonal cycle with the breakdown, usually, of the halocline in autumn and its re-stablishment in spring. There were the dilution effects of rainfall and the heating effects of prolonged periods of sunshine. Yet when the conditions prevalent in Holkham Bay are compared with those in the Salts Hole, these effects only underline how little variation occurs in the latter. Most estuarine environments are typified by variability and exposure to change. The water in an estuary is radically changed by the flow and ebb of the tide, twice daily, and concomittant changes in salinity, temperature, oxygen supply and the ingress of marine predators. The deposition of sediments leads to mud flats and for an animal to colonize an estuary it must usually be capable of living in, or near to muddy deposits; and it must be able to adapt to a wide range of environmental fluctuations, especially the variable salinity and temperature. In addition the turbidity of the water may discourage settlement. Hence both the nature and variability of environmental factors present major problems to the colonization of estuaries by animals from adjacent fresh-water or marine habitats, leading to a fauna usually few in species although often abundant in numbers. By far the major contrast that leads to the stability of conditions in the Salts Hole is the absence of tidal flow. The fauna is constantly submerged and consequently largely protected from both the temperature and salinity changes to which estuarine animals are exposed. The conditions are much more paralleled by a sub-littoral habitat than by an estuarine habitat, yet the fauna is essentially estuarine in nature. The substratum is also atypical of estuaries. Because there is no deposition of silt from the sea, most of the bed of the Salts Hole is a mixture of gravel and fine sand; turbidity is never a problem either for settlement or for gill-breathing animals.

The hydrographic survey established that during the period of the study there was little change in the monitored conditions. Salinity, temperature and oxygen concentrations have been monitored at, at least two stations on a $\pm$ monthly basis, for eight years to the present time. No fluctuations outside the range of the 1974-77 survey have been record-

ed for these environmental factors. Pantin's data recorded in Hunt (1971) from the years 1962-63 are also within this range. To extend these observations to cover long-term fluctuations, if any, is of course purely speculative. These studies have shown, however, that despite profound differences in air temperature and rainfall, over this period of time, the incoming water at the spring is remarkably constant, due presumably to the large dimensions of the reservoir under the sands of Holkham Bay. It is hard to see how this situation could have been altered much during the two hundred and fifty years of the Salts Hole's existence without leading to a drastic change in the size, shape and nature of the pond. The map of 1843 shows a pond of similar outline to the present, and it is known that water has flowed through the pond since at least 1742 because there are records of the maintenance of the culvert from that date.

Given that the environmental stability of the Salts Hole exceeds that of the neighbouring salt marshes, can the same be said of the stability of trophic resources?

Unfortunately no studies of primary or secondary production were undertaken, but on the evidence of zooplankton population fluctuations, the Salts Hole follows a regular cycle of spring and autumn blooming of the type associated with eutrophic lakes.

In such a small pond, this is at first sight very surprising since it is clearly too shallow to develop a typical pattern of thermal stratification. However there is good evidence of a halocline which is maintained until autumn and this may lead to water deficient in nutrients remaining on the surface of the denser and presumably nutrient-rich-water below. This last assumption was not tested but there seems to be no reason why sea-water, in its passage through the sand to the pond, should lose substantial amounts of nitrates or phosphates.

There is also no evidence that the pond shows a degree of trophic-resource-stability greater than that of Holkham Bay. In temperate regions, it is largely climate which controls the level of primary production and sea temperatures would certainly be no less favourable than pond temperatures in maintaining phytoplankton levels. Much of the macrofauna of estuaries feeds on detritus which is produced outside the ecosystem. This is deposited, together with silt, (whose origin is also largely marine), when the velocity of the incoming currents are reduced. This means that for detritivores, estuaries are often resource-stable environments because the detritus can accumulate from even relatively short periods of primary production. Clearly the Salts Hole

cannot acquire quantities of detritus from outside sources, although it may acquire material blown in from the sand-dunes and salt marshes. A detritivore such as C. volutator frequently exceeds densities of $10^4/m^2$ in estuaries but its estimated density is only $2 \times 10^2/m^2$ in the Salts Hole; this is consistent with the absence of large deposits of detritus.

There is therefore no evidence to suggest that in terms of resource-stability, the Salts Hole is more stable than the surrounding salt-marshes. Indeed, the opposite situation seems to be more likely. If either of the two hypotheses predicting relationships between variability and environment or resource stability are applied to the Salts Hole then both predict lower levels of genetic variation. However, in order to achieve this, both hypotheses predict that natural selection must take place.

In a genetically isolated species there are only two processes, acting separately or in concert, that can account for genetic change. The first process is random genetic drift; in the absence of selective differences among genotypes, the sampling process forces a departure from the status quo. The dynamics of this process have been described especially by Wright (1969) and by Crow and Kimura (1970). The second process is, of course, selection.

The suggestion that in general two or more alleles co-existing in natural populations are adaptively equivalent or indistinguishable (i.e. neutral with respect to one another) has been called the neutral hypothesis. This important hypothesis has been tested in two macromolecular fields; aminoacid sequence in proteins and nucleotide sequence in nucleic acids. The predictions of the neutral hypothesis are formulated in terms of random genetic drift.

Decisive evidence (Salser and Isaacson, 1976, Grunstein et al, 1976), has been obtained that certain alternative bases in the third position of codons are indeed adaptively equivalent. Thus the neutral hypothesis is correct in at least one vastly important molecular domain.

On the other hand the evidence based on studies of Kimura (1976) and Milkman (1976) now appears to show that the neutral hypothesis does not generally hold for protein variation. That is to say, most allozymic differences are potentially adaptively significant. Ohta (1974) points out, however, that low selection coefficients will be ineffectual in small populations, rendering the corresponding genotypes effectively neutral. In order to counteract random genetic drift, the required value of s is in the order of $\frac{1}{Ne}$, where Ne is the effective population size. (As generally stated, the requirement is that $s \ll \frac{1}{4Ne}$).

Accordingly, in order to ascertain with certainty the relative importance of selection and random genetic drift, the following variables must be estimated with considerable accuracy; the effective size of the population undergoing selection, the possible magnitude of selection coefficients and the effective number of alleles at each locus. This has in practice only been possible to carry out in controlled laboratory conditions on animals whose genotypes are known, such as Drosophila melanogaster (Johnson and Powell, 1974).

Population estimates of the three crustacean species studied in the Salts Hole, I. chelipes, G. duebeni and P. flexuosus indicate that the numbers of adults in all three are relatively small. In a pond whose total surface area is only 5.2×10^3 m^2 this is hardly surprising. Accordingly it is to be expected that variation in these populations has largely been the result of random genetic drift, because selection coefficients of some magnitude would be required to overcome the random effects.

I expected that the studies of the effects of combined environmental changes on survival would confirm that any variation recorded in the pond populations would be of a random nature. This was not the case. There are many differences recorded between the Salts Hole and Bay populations. These specific differences have already been referred to. Essentially they show that genetic adaptation has taken place, but give no clue as to its cause. However, what is clearly demonstrated in figs. 17, 20 and 23 is that for all three species the response surface centres of the Salts Hole populations correlate more closely to the salinity and temperature means of their own habitat than do the Bay samples. On one occasion only among the 11 possible pairings - a Bay sample is closer to the mean salinity of the pond than its equivalent Salts Hole sample (I. chelipes 8 mg/l O_2). Twice the same is true for Bay samples and the mean temperature value (G. duebeni 8 mg/l O_2, P. flexuosus 5 mg/l O_2). The chance that the Salts Hole samples are closer to these means in 10 cases out of 11 for salinity and 9 out of 11 for temperature may be computed using the product rule, as 1 : 40. This distribution is clearly unlikely to be achieved by chance. The only alternative hypothesis is that selection has taken place.

If the precautions to exclude acclimation effects were sufficient then such physiological differences recorded between the Salts Hole and Bay populations not due to sampling error, must be due to genetic adaptation.

That there is a high degree of genetic variability in all three

species is supported by electrophoretic studies of the Mdh and Lap isoenzymes. The amount of genetic variability, however maintained (e.g. by heterosis, frequency-dependent selection and selection in heterogeneous environments), is regarded by most evolutionists as adaptive, but the significance of this variability in relation to the environment is yet to be established. It has been proposed that high genetic variability permits the exploitation of a wider range of habitat resources and a consequent expansion of the ecological niche (Van Valen, 1965). If the population is nearly panmictic the niche will be broadened and a continuous array of genotypes will occupy continuously intergrading microhabitats. If gene flow is low, the niche will be subdivided and individual populations will express only a fraction of the total variability. In the latter case gene flow might impose a genetic load on populations. Genetic variability may be generated through differential selection over the heterogeneous environment or it may be the response to competitive pressure among contiguous individuals of a species, (Antonovics, 1971), the intraspecific counterpart of the competitive exclusion principle. An alternative hypothesis of genetic variability states that much of it is an artifact of gene flow (Soulé, 1971). According to this gene flow hypothesis,some of the genetic variability would not be specifically adaptive unless high genetic variability per se is adaptive and migration rates are selectively adjusted to keep it high.

The concept of the intrinsic adaptiveness of high genetic variability is often cited by evolutionists, usually without recourse to causal explanations of the origin of variability. The core of the argument is that high genetic variability is a form of insurance against environmental perturbations. Among the multitude of genotypes arising in each generation from recombination and syngamy there should exist combinations potentially adaptive over a wider range of environmental conditions than the population actually experiences. Under a new environmental stress the appropriate alleles are available and enable a fraction, at least, of the population to survive and propagate. The 'price' for this insurance is exacted each generation as a certain number of non-adapted genotypes. This 'genetic load' may, in fact, be spurious because genotypes with a low fitness may comprise most of the inevitable prereproductive deaths, the 'ecological load' of each generation.

The Salts Hole provides an environment in which gene flow is very low or non-existent. In the light of either of the hypotheses discussed above, this population should express a smaller portion of the total variability available to the species, than where gene flow

is higher. Studies on the Lap and Mdh enzymes of the crustaceans in the Salts Hole indicate that there are indeed fewer alleles recorded here than at any other station. It must be emphasised, however, that this is the conclusion of a far from comprehensive survey and examination of a wider range of enzymes than proved possible here, should be undertaken. Whether this absence of alleles is best explained in terms of gene extinction due to random genetic drift or to the selection of more advantageous alleles, with migration of these alleles from surrounding populations prevented, extinction is more likely to occur. It is tempting to speculate that such small populations even given relatively high levels of physical and biotic stability would have been subject to periods when numbers were very low indeed. These conditions would serve as 'size bottlenecks' which would promote random genetic drift.

Had widespread deletion of alleles influencing potential adaptation to a wider range of environmental conditions taken place, it would surely have been observed as marked stenoplasticity in the Salts Hole populations, when the species were subjected to physiological stress experiments. There is no evidence of this from any species with the possible exception of G. duebeni at 8 mg/l O_2. In the case of P. flexuosus, it is the Bay population which is less tolerant of the environmental extremes than the Salts Hole population. Only in the location of response surface centres is there clear evidence of genetic adaptation. That this selection has been brought about by virtue of trophic stability is clearly unlikely because there is no evidence that the Salts Hole is less affected by seasonal change than the surrounding marshes. Other environmental variables besides stability of trophic resources can be identified, that correlate well with the levels of genetic variation observed in many species studied, especially those species of deep sea environments. A possible one might be the abundance of trophic resources. In order to decide between the two hypotheses - abundance versus stability of trophic resources - an environment can be chosen where the trophic supply is stable but scarce. The deep sea, of course, meets this requirement. As Hessler and Jumars (1974) have demonstrated, the deep sea is amongst the most temporally stable environments on earth, both in terms of physical parameters and supply of trophic resources. Seasonality seems to be nearly or completely absent, and deep sea animals depend on the organic remnants reaching them through the water column, except in the rare 'hot spots' where autotrophic bacteria, utilizing hydrogen sulphide and methane, are the basis of food webs.

Most of the research mentioned above concerning studies on deep water species - (Ayala et al, 1975b, Gooch and Schopf, 1972) have shown these species are genetically highly polymorphic which is consistent with the hypothesis proposing that high levels of genetic variation correlate with stable resources. The results are inconsistent with the hypothesis proposing a correlation between abundance of trophic resources and high levels of genetic polymorphism. The hypothesis applied to the Salts Hole, with its low levels of trophic resources and lower levels of polymorphism is supported, but its validity is questionable as the studies on the deep sea have indicated. However, it has been argued by Stenseth (1979) that the abundance of trophic resources is instrumental in determining the number of species present in any habitat. He applied the Red Queen Hypothesis to both current as well as geological time. This hypothesis asserts that any gain in fitness by one species is balanced by an equal and opposite loss in fitness of all other interacting species within the community. As pointed out, for instance by Janzen (1968) and Lawlor and Maynard Smith (1976), two time scales must be considered in evolutionary ecology; the ecological or current, and the evolutionary or geological time. Ecological and evolutionary stability correspond to these two time scales. Similarly there are two types of extinction - those operating in ecological time can be designated as local extinction. This is a repeatable phenomenon in the sense that one species may become extinct in several habitats but continuesto exist in others. Extinction operating in evolutionary time is global extinction. This is an irreversible phenomenon and represents the termination of a taxon's existence. For both time scales, it is reasonable to assume that the extinction rate for a given population will increase with increasing amplitude of its fluctuations in population density, as Leigh (1975) postulated. Further this rate, according to McArthur and Wilson (1967), will increase with decreasing population density of the species. The Red Queen Hypothesis treats evolution as ecologically more than genetically controlled, in that ecology is the driving force, not a bystander or boundary-maker. In this context genetics represents the boundary maker. According to Van Valen (1977) the hypothesis is most easily explained by equating realised fitness with each individual's control of a constant amount of the trophic energy available to a group of related and competing species. The smaller the trophic resource available, the

fewer the species that can survive in the habitat.

One hundred and sixty species of animals have been recorded from the vicinity of Scolt Head Island (Pantin et al, 1960). Clearly the habitats present there are diverse. If organisms specifically adapted to mud-banks, salt-pans and the like, are excluded, some seventy species of animals are left that are known to survive in habitats in which the environmental parameters approximate to the conditions found in the Salts Hole. Only twenty nine of these species have been observed in the Salts Hole. What is perhaps more interesting is that groups of species which are known to occupy closely related niches have been replaced by a single species in the Salts Hole. Six species of Nereidae are recorded at Brancaster, but only N. diversicolor in the Salts Hole. Of nine species of detritivorous polychaetes only A. marina is present. Fourteen species of suspension feeding bivalves are present in Brancaster Bay, but only one(C. glaucum) in the Salts Hole pond. Certain detritivores, for example, Carcinus moenas L. are common inhabitants of salt-pan and shore alike, but are noticeable in their absence in the pond. Frequently three or even four gammarid species may be collected from a single vicinity, for example, at Norton Creek, but only G. duebeni is found in the Salts Hole. Recently Kolding (1981b) has suggested that G. duebeni is adapted specifically to brackish-water pools.

The conclusion to be drawn from this is not, of course, that each species does not have a defined ecological niche, but rather that when the level of trophic resource is low, there may not be sufficient to support all the specialists and only those species able to exploit the broadest resource bands will be present. The results from the Salts Hole accordingly correlate with the predictions of the Red Queen Hypothesis.

The Holkham Salts Hole was undoubtedly once part of a creek system that existed behind the sand-hills as a tideway into the salt-marshes before these were enclosed by artificial embankments to become the grazing fields they are today. There is little doubt that the main drainage creek shown in a map of 1590 preserved in the muniment room of Holkham House, is the channel of which the mouth was eventually to form the Salts Hole. It has not been possible to establish more precisely when the Salts Hole came into being, but the process of conversion from creek to pond must have been taking place during the seventeenth century. Biological isolation cannot have occurred until after the 1719/20

embankments were built. Once the sea-wall was in existence, the pond would have finally been cut off from comminication with the sea except in conditions of flooding extreme enough to breach or overflow the embankments. These conditions occur only very rarely and in fact only one such flood is known to have entered the pond. On 31 January 1953 a great storm and sea surge coincided with a very high tide to cause disastrous flooding in Britain and the Netherlands. The embankments east and west of the Salts Hole both failed to contain the flood and sea-water reached the pond from both directions. According to information obtained locally, sea-water remained above the level of the pond for several days so that marine organisms could have been brought to the pond by this means. A storm in 1828 is known to have breached the walls near Wells, but there is no evidence that waters actually reached the pond. A storm in 1974, sufficient to lift a cargo-boat from Wells Harbour onto the sea road, and to break through the sea defences in several places, did not flood the Salts Hole. It is not possible, therefore, to exclude the possibility of floodings in earlier years, but they would appear to be very rare events. Hunt (1971) favours the idea that the Salts Hole fauna is in the main a relic from some 250 years ago when the pond was part of a tidal creek system.

A lagoon similar to the Salts Hole existed at Titchwell (TF765448). Formed when the North Sea breached the sand dunes in 1938 and flooded a small area of farmland, it remained isolated until 1969 when a breach appeared in the western sea-wall. Williams (1972) who described its fauna, points out the remarkable similarity between it and that of the Salts Hole. Another, and larger pond, known as Abrahams Bosom, is still in existence. Situated behind the dunes at Wells (TF912454), it maintains a more or less constant salinity (15-20‰) and a restricted but similar fauna to the Salts Hole. Its use as a childrens boating lake and close proximity to a caravan site has had serious effects on its fauna and also presents problems of access for regular monitoring.

The overall similarity of the fauna of these three ponds reinforces the suggestion that the Salts Hole maintains a largely relict fauna comprising mainly estuarine species. Studies on the genetic divergence of the crustaceans studied clearly show that the Salts Hole possesses the most divergent population for all three species studied. This is particularly noticeable in I. chelipes. Is it surprising that significant changes have taken place in such a short period of time? Consideration will be first given to the effects of random genetic

drift. The population, due to the limited trophic resources available in the pond will always have been sparse in numbers. Seasonal influences will presumably have determined that the supply of primary resources on which I. chelipes directly feeds, will have been variable. The populations may well have gone through several 'bottlenecks' during 250 or so years. Labourg (1971) demonstrated that two broods per year may be expected for I. chelipes in temperate waters, thus permitting a maximum of about 500 generations. The same could be true of G. duebeni, based on the evidence of Kolding and Fenchel (1981). Assuming that two alleles have an equal frequency, Crow and Kimura (1970) have shown that the chances of one gene becoming extinct in 500 generations is 0.001 unless the population drops below 2,500. There is of course no reason to assume that the initial frequency of any allele lost approached 0.5, but clearly a population size of 2,500 is very small for a grazing species and is 50 times less than the present population. It should also be noted that the present frequencies of some of the alleles do differ by nearly 50% between populations of I. chelipes (Mdh - 3^{d}). Similar differences may be noted in G. duebeni (Mdh - 2^{c}) and P. flexuosus (Mdh - 3^{a}). If random genetic drift seems unlikely, selection coefficients of sufficient magnitude to overcome drift seem even less likely, But this assumes that all populations are similar until isolation takes place, which is clearly not supported by the electrophoretic studies carried out here - or by any other workers. Smith (1980) pointed out that heterogeneity exists in all environments. Zones of temperature or of salinity for example, bring about ecological isolation even before geographical isolation takes place. 'Pre-Salts Hole' populations may have shown high levels of divergence within the tideway prior to their eventual geographical isolation. This might be said to be the case in the Blakeney Point populations, similarly restricted to a tideway. They also show higher levels of genetic divergence between themselves and the next station, Wells, than do populations from the first three stations.

Populations of several species of crustaceans, although not large, have been of sufficient size to permit selection to overcome the effects of random genetic drift in a measure of genetic adaptation to the peculiar environmental conditions of the pond. There is reason to believe this adaptation may be taking place in other species eg. Cordylophora lacustris Allman, which is intolerant of the salinities in which typical hydroids are found.

To summarise the arguments presented above, in the knowledge that selection is taking place, although it may not be overriding the effects of drift in all cases, it is possible to try to equate the levels of genetic polymorphism with other characteristics. Lower levels of polymorphism have been recorded in the pond than any of the other stations on the north Norfolk coast. Levins' environmental stability hypothesis predicts that selection leading to low levels of genetic polymorphism proceeds in stable environments. It was established that the Salts Hole maintains remarkably stable levels of those environmental variables (salinity, O_2 concentration and temperature) which usually influence marine populations.

There therefore seems to be evidence to support this hypothesis in these studies.

Valentine's trophic-resource-stability hypothesis predicts that low levels of genetic polymorphism would be selected in habitats where there was a cyclic or irregular supply of trophic resources. This would be true of the Salts Hole as it is true of the other stations on the north Norfolk coast, but why should that irregularity be more pronounced in the pond?

There is evidence that abundance of trophic resources may influence the detritivore diversity in the Salts Hole. Detritus resources are generally meagre here. The detritivores are at the mercy of the primary producers of the pond alone, for unlike estuarine systems, there can be no imput of material from outside other than that blown off the dunes and salt marshes. A year of poor primary production will influence the pond more than at the other stations. Even grazing species may be influenced more in the Salts Hole because phytoplankton production in the sea is often patchy but tidal movements equalize distribution. This situation is not applicable to the pond. Given the reservations already stated then there is some correlation in the pond between the levels of genetic polymorphism and the trophic-resource-stability hypothesis.

Relatively few species make up the Salts Hole fauna and there are similarities with species found in similar salt-water lagoons on the Norfolk coast, all of which show a predominently estuarine fauna. The Red Queen hypothesis predicts that fewer species should survive in habitats where there are lower levels of trophic resources. The data of the Salts Hole support this hypothesis.

The studies outlined here are only preliminary experiments which have utilized a remarkable resource. It is a resource which demands further attention, because it presents an ideal testing ground for hypotheses which have great bearing on the mechanisms of evolution.

The last words must be from Carl Pantin, discover and 'champion' of the Salts Hole (Pantin 1965).

"Accidental circumstances have given rise to an almost ideal 'Natural aquarium' for the preservation of marine organisms: one which would have cost great sums of money to build artificially; one which contains a 'built-in' mechanism for the maintenance of constancy of conditions..... one which offers unlimited possibilities for ecologist and evolutionist alike."

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APPENDICES

TABLE OF CONTENTS

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APPENDIX ONE

SUBSTRATUM ANALYSIS.

METHODOLOGY AND EXAMPLES FROM FOUR STATIONS.

The methodology of preparing samples was similar to that outlined in Buchanan and Kain (1971). After hand sorting for benthos, two subsamples of substratum were returned to the laboratory for processing. These were oven dried and from them 100g of material was removed and processed by passing through a series of 13 sieves in an Endecott shaker. The weights of the graded sediment were taken. Two subsamples were processed for each station.

Where large pebbles or shells made up a substantial part of the sediment, these were processed separately using extra-wide-meshed sieves.

A few stations had substantial quantities of silt and clay in the sediment and these were analysed using pipette analysis techniques also described in Buchanan and Kain (1971).

The sieve mesh grades were converted into phi notation and cumulative frequency curves were plotted for all stations.

Four examples of these are included here, one each for the four general types of substratum recorded - viz. gravel, coarse sand, fine sand and silt. From the cumulative curves it is easy to gain measures of central tendency, degree of scatter and degree of assymmetry of sample.

The median diameter is a good measure of central tendency which can be determined easily from the cumulative curve by reading the phi value which corresponds to the point where the 50 per cent line crosses the cumulative curve. It is defined as the phi value which is larger than 50 per cent of the phi values in the distributions and smaller than the other 50 per cent. It should be abbreviated as $Md\phi$.

Although the median diameter gives an average value, it represents a central point but does not indicate the degree of spread of the data about this central tendency. A second measure, the quartile deviation, measures the number of phi units lying between the first and third quartile diameters, that is, between the 25 per cent and 75 per cent points on the cumulative curve where $QD\phi = (Q_3\phi - Q_1\phi)/2$. A sediment with a small spread between the quartiles is regarded as being 'well sorted'.

The quartile deviation, which measures the spread, does not give any indication of the symmetry of the spread on either side of the average. If there is a tendency for the data to spread on one side more than the other, this asymmetry is called skewness. The phi quartile skewness may be calculated from the equation $Sk_q\phi = (Q_1\phi + Q_3\phi)/2 - Md\phi$. A positive value will indicate that the mean of the quartiles lies to the right of the $Md\phi$ and should be prefixed +, while a negative value would lie to the left and should be prefixed - to indicate negative skewness.

US STANDARD MESH	Ø	SAMPLE 1. % wt	SAMPLE 2 % wt	MEAN % wt	CUMULATIVE %
5	-2	7·89	8·93	8·41	8·41
7	-1·5	3·12	3·61	3·37	11·78
10	-1·0	4·73	4·94	4·84	16·61
14	-0·5	4·29	4·38	4·34	20·95
18	0	3·64	3·73	3·69	24·63
25	0·5	4·83	4·61	4·72	29·35
35	1·0	10·72	10·02	10·37	39·72
45	1·5	4·37	4·39	4·38	44·10
60	2·0	5·78	5·54	5·66	49·76
80	2·5	10·21	9·83	10·02	59·78
120	3·0	17·55	18·00	17·78	77·56
170	3·5	13·04	12·81	12·93	90·48
230	4·0	9·83	9·21	9·52	100·00

STATION D3

'FINE SAND'

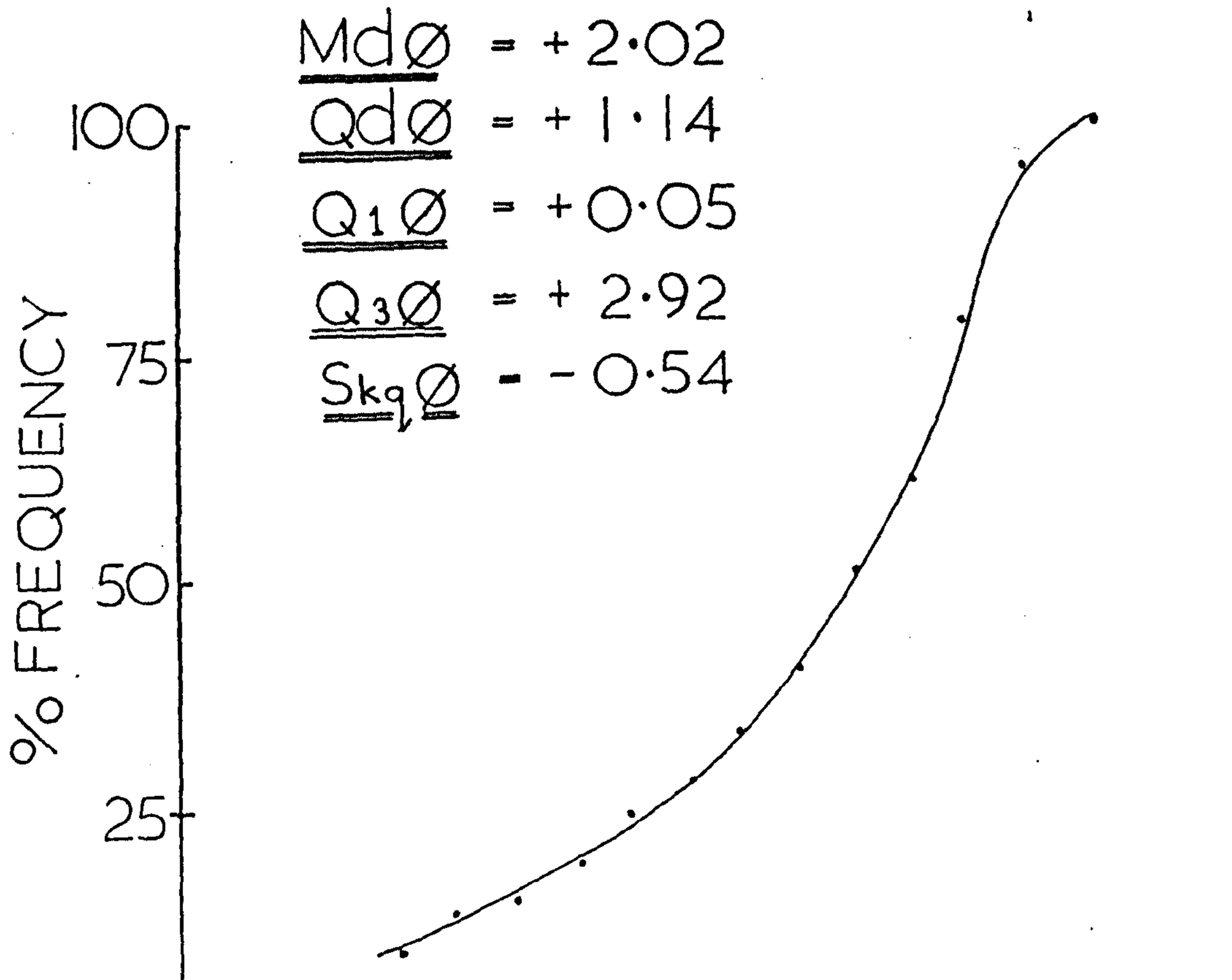
$\underline{Md\emptyset} = +2{\cdot}02$

$\underline{Qd\emptyset} = +1{\cdot}14$

$\underline{Q_1\emptyset} = +0{\cdot}05$

$\underline{Q_3\emptyset} = +2{\cdot}92$

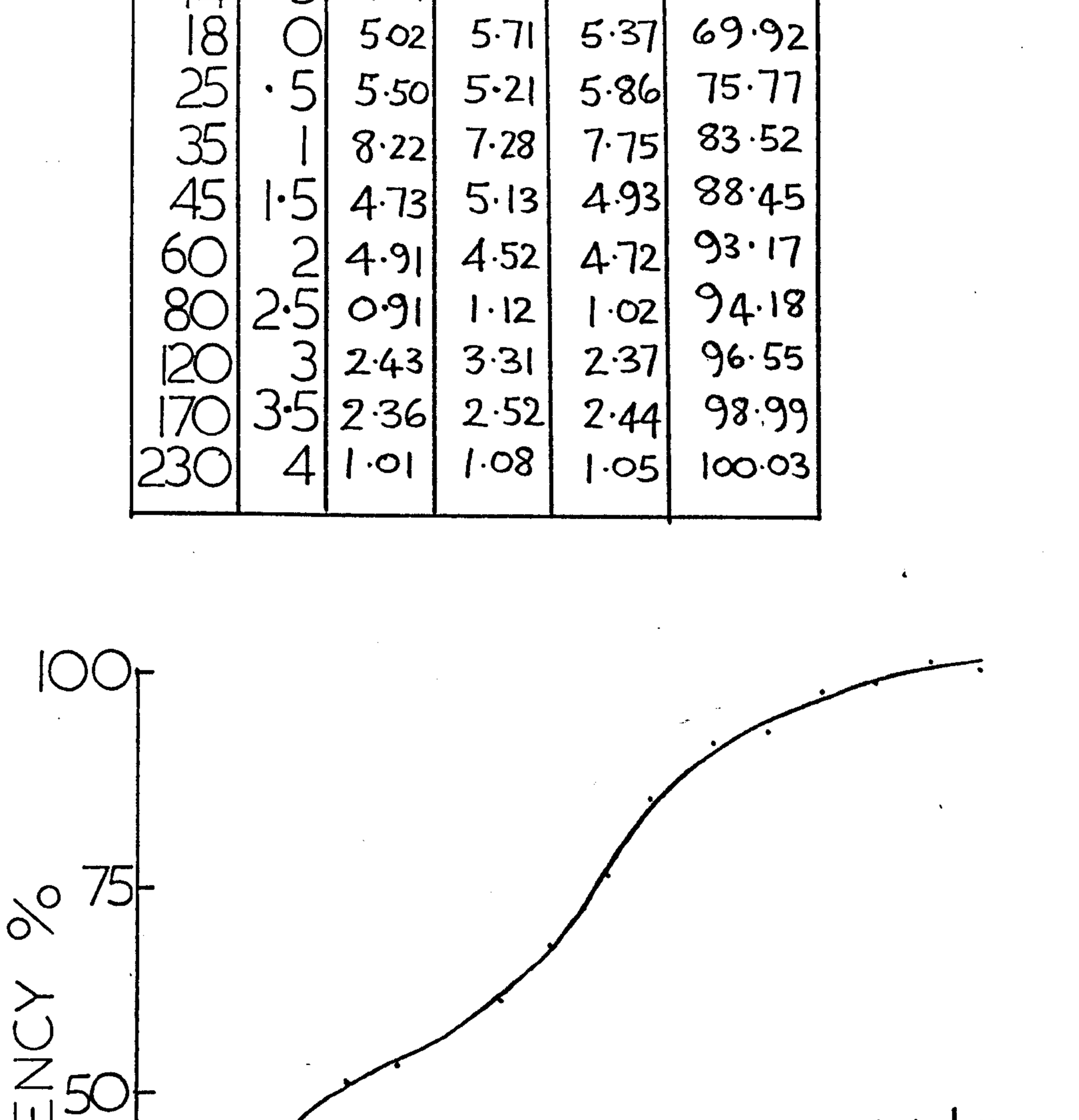
$\underline{Sk_q\emptyset} = -0{\cdot}54$



STATION E6

'GRAVEL'

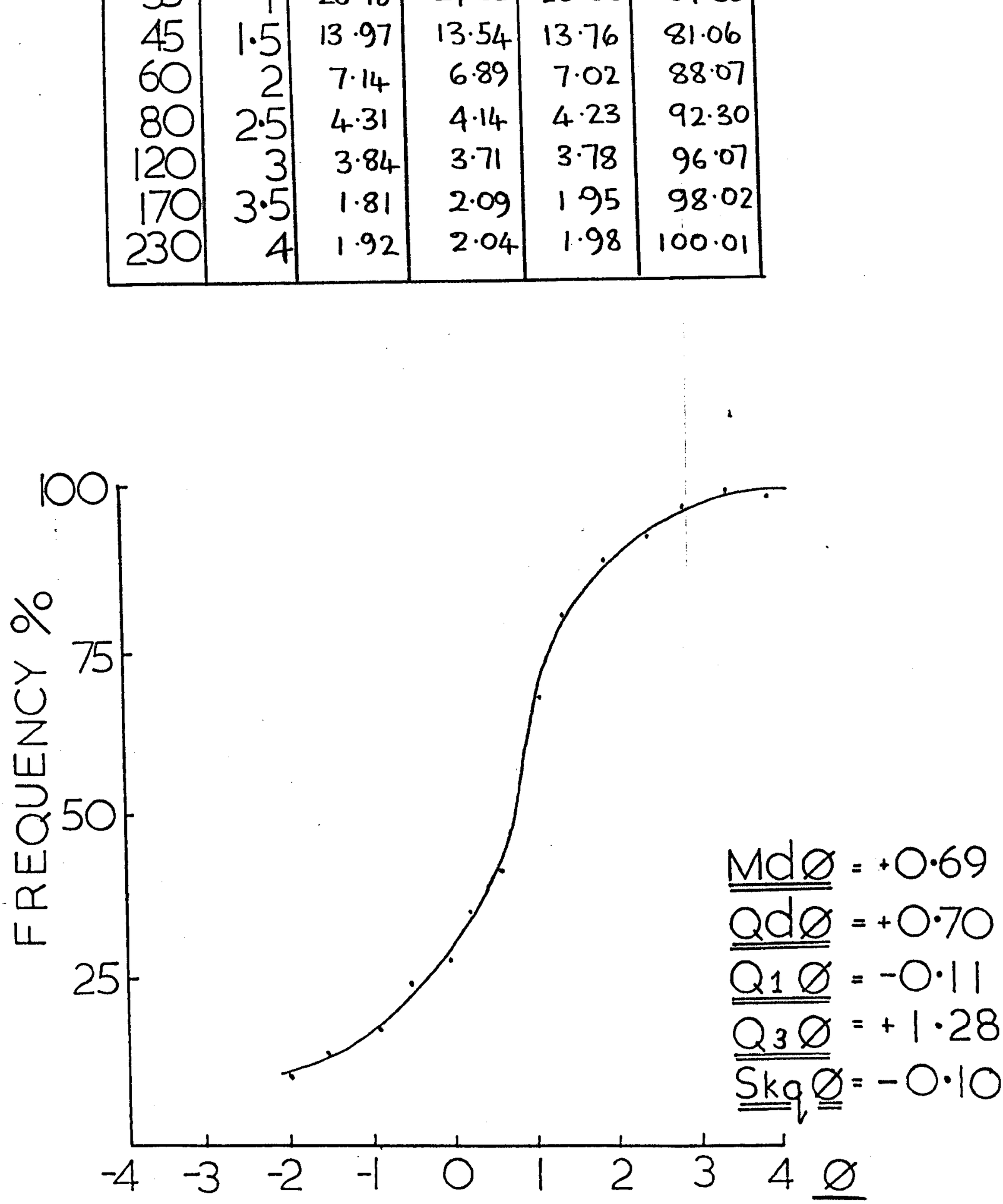
U.S. MESH	Ø	SAMPLE 1 % wt.	SAMPLE 2 % wt.	MEAN % wt	CUMULATIVE %
—	-4	Pooled		8·91	8·91
—	-3	Pooled		7·45	16·36
5	-2	24·93	22·54	32·65	49·01
7	-1·5	3·91	3·72	3·82	52·81
10	-1	5·73	6·38	6·06	58·88
14	-·5	4·94	6·41	5·68	64·55
18	0	5·02	5·71	5·37	69·92
25	·5	5·50	5·21	5·86	75·77
35	1	8·22	7·28	7·75	83·52
45	1·5	4·73	5·13	4·93	88·45
60	2	4·91	4·52	4·72	93·17
80	2·5	0·91	1·12	1·02	94·18
120	3	2·43	3·31	2·37	96·55
170	3·5	2·36	2·52	2·44	98·99
230	4	1·01	1·08	1·05	100·03



MdØ = -1·75
QdØ = +1·50
Q_1Ø = -2·58
Q_3Ø = +0·42
SkqØ = +0·67

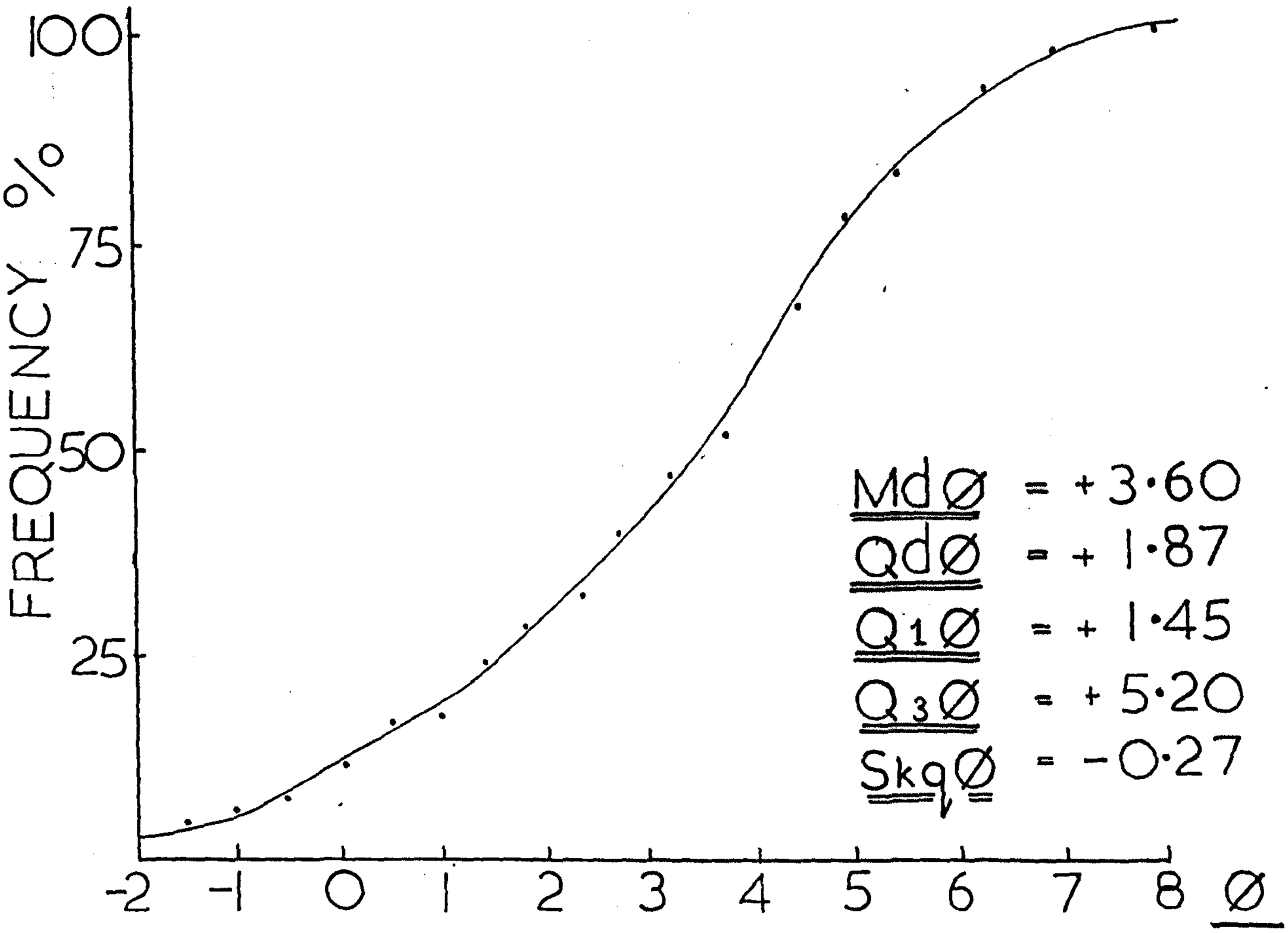
STATION F3 'COARSE SAND'

U.S. MESH	Ø	SAMPLE 1 %wt	SAMPLE 2 %wt	MEAN % wt	CUMULATIVE %
5	- 2	7·27	8·92	8·09	8·09
7	- 1·5	4·44	4·38	4·41	12·50
10	- 1	3·87	4·14	4·01	16·51
14	- ·5	4·14	3·97	4·06	20·56
18	0	6·30	6·19	6·25	26·81
25	·5	12·21	12·67	12·44	39·25
35	1	28·78	27·33	28·06	67·30
45	1·5	13·97	13·54	13·76	81·06
60	2	7·14	6·89	7·02	88·07
80	2·5	4·31	4·14	4·23	92·30
120	3	3·84	3·71	3·78	96·07
170	3·5	1·81	2·09	1·95	98·02
230	4	1·92	2·04	1·98	100·01



STATION J4 'SILT'

U.S. MESH	Ø	SAMPLE 1 % wt	SAMPLE 2 % wt	MEAN % wt	CUMULATIVE %	
5	- 2	3·20	6·36	4·78	4·78	
7	-1·5	2·72	2·48	2·60	7·38	
10	- 1	1·04	0·97	1·01	8·39	
14	- ·5	2·16	1·89	2·03	10·41	
18	0	3·74	3·61	3·68	14·09	
25	·5	3·34	3·02	3·18	17·27	
35	1	5·09	4·78	4·94	22·20	
45	1·5	2·93	2·86	2·90	25·10	
60	2	3·72	3·64	3·68	28·78	
80	2·5	5·32	4·99	5·16	33·93	
120	3	8·92	8·61	8·76	42·69	
170	35	6·72	6·54	6·63	49·32	
230	4	6·36	6·17	6·27	55·59	
—	5	16·23	16·43	16·33	71·92	PIPETTE ANALYSIS
—	6	13·61	12·95	13·28	85·20	
—	7	10·32	10·02	10·17	95·37	
—	8	4·59	4·68	4·64	100·00	



APPENDIX TWO

HYDROGRAPHIC AND CLIMATOLOGICAL DATA

1974 - 1977

a) Salinities and monthly rainfall

b) Water temperature and air temperatures

c) pH and O_2 concentration.

a) <u>Salinities and monthly rainfall</u>

Salinity of water recorded at:-

Station A - from spring

Station B - from culvert

Rainfall recorded at Cromer DCNN station 3069.

YEAR	MONTH	SALINITY ‰ STATION A	STATION B	MONTHLY RAINFALL mm
	APR	25.0	21.0	5
	MAY	24.1	20.5	13
	JUNE	22.6	22.8	29
1974	JULY	24.2	23.5	63
	AUG	22.6	21.9	94
	SEPT	23.1	21.8	59
	OCT	24.4	18.4	134
	NOV	22.2	20.6	90
	DEC	22.4	19.8	27
	JAN	23.9	20.4	69
	FEB	21.9	18.8	20
	MAR	23.7	20.3	67
	APR	25.3	21.7	51
	MAY	24.0	18.5	45
1975	JUNE	22.2	20.4	35
	JULY	23.4	21.6	48
	AUG	21.7	22.6	4
	SEPT	22.9	21.5	79
	OCT	22.0	20.6	39
	NOV	25.5	21.9	59
	DEC	23.6	20.7	37

YEAR	MONTH	SALINITY ‰ STATION A	STATION B	MONTHLY RAINFALL mm
	JAN	25.5	24.2	65
	FEB	26.3	26.3	10
	MAR	25.4	23.5	19
	APR	25.4	21.0	18
	MAY	24.8	22.5	36
1976	JUNE	26.0	23.9	6
	JULY	27.2	22.5	40
	AUG	26.8	22.9	60
	SEPT	25.8	20.4	75
	OCT	28.4	23.2	126
	NOV	28.0	23.2	60
	DEC	26.1	22.3	60
	JAN	26.6	20.6	92
	FEB	24.9	21.3	104
	MAR	23.2	19.2	33
1977	APR	23.7	20.0	35

b) Water temperature and air temperature

Water temperatures recorded at

Station A - m from spring

Station B - m from culvert

Maximum and minimum means of air temperatures recorded at Cromer: DCNN station 3069.

YEAR	MONTH	WATER TEMPERATURES °C		MEAN AIR TEMP.°C	
		Station A	Station B	Max	Min
	APR	7.0	7.0	9.1	4.6
	MAY	7.7	7.1	15.5	6.8
1974	JUNE	7.9	8.9	16.9	9.6
	JUL	8.8	11.0	17.3	10.4
	AUG	9.0	10.2	19.9	12.2
	SEPT	9.9	11.2	16.7	9.1
	OCT	10.9	9.2	10.7	6.1
	NOV	10.8	7.9	9.2	4.1
	DEC	10.4	6.8	10.5	4.8.
	JAN	7.0	7.0	9.3	3.8
	FEB	7.1.	7.1.	8.1	2.3
	MAR	8.2.	8.8	7.1	2.5
	APR	8.0	8.8	11.5	4.5
	MAY	7.9	10.5	12.2	6.7
1975	JUN	8.2	11.1	17.6	9.7
	JUL	9.2	12.3	21.1	13.2
	AUG	9.3	12.6	22.9	14.6
	SEP	10.1	12.0	18.5	10.9
	OCT	11.0	10.4	13.6	7.8
	NOV	10.9	7.6	9.3	4.2
	DEC	9.5	7.0	7.6	3.0

	JAN	9.5	7.0	8.1	3.1.
	FEB	9.2	8.0	7.1	2.2
	MAR	9.2	8.2	7.7	2.1
	APR	8.4	11.0	11.1	4.8
	MAY	7.8	10.1	15.9	7.8
1976	JUNE	7.3	12.4	23.3	12.3
	JUL	8.0	12.2	21.9	14.1
	AUG	8.6	12.8	20.3	14.2
	SEPT	9.0	10.1	17.4	11.7
	OCT	9.2	10.2	14.2	9.0
	NOV	9.6	8.2	9.7	4.6
	DEC	10.0	7.0	5.3	0.9
	JAN	9.6	6.7	5.2	1.1
	FEB	9.5	6.4	7.4	2.3
1977	MAR	9.0	7.5	10.6	3.6
	APR	8.9	8.7	11.3	3.6

c) pH and O_2 concentration

Both pH and O_2 concentration recorded at

Station A - m from spring

Station B - m from culvert.

YEAR	MONTH	pH		O_2 concentration mg/l	
		Station A	Station B	Station A	Station B
	APR	7.42	8.05	0.40	10.50
	MAY	7.40	8.08	0.35	10.55
1974	JUN	7.38	7.88	0.40	11.25
	JUL	7.40	8.24	0.35	10.85
	AUG	7.40	8.00	0.35	10.40
	SEPT	7.35	7.86	0.35	10.35
	OCT	7.40	8.14	0.25	10.40
	NOV	7.35	7.87	0.25	10.45
	DEC	7.20	8.20	0.35	9.80
	JAN	7.40	8.05	0.50	9.75
	FEB	7.30	8.22	0.55	10.30
	MAR	7.30	8.24	0.35	10.60
	APR	7.35	7.98	0.60	10.45
	MAY	7.35	7.90	0.70	10.60
1975	JUN	7.44	8.00	0.45	10.40
	JUL	7.35	7.75	0.25	9.80
	AUG	7.32	7.80	0.30	10.50
	SEPT	7.34	7.72	0.25	10.25
	OCT	7.34	7.90	0.45	10.50
	NOV	7.40	7.73	0.25	10.30
	DEC	7.42	7.68	0.45	10.50
	JAN	7.33	7.74	0.40	9.85
	FEB	7.42	7.62	0.30	10.30
1976	MAR	7.33	7.82	0.45	10.85

	APR	7.34	7.70	0.35	10.60
	MAY	7.33	7.88	0.40	10.30
1976	JUNE	7.40	7.76	0.35	10.50
	JUL	7.43	7.85	0.50	10.80
	AUG	7.39	7.95	0.55	10.60
	SEPT	7.38	8.15	0.60	10.40
	OCT	7.35	8.03	0.50	10.10
	NOV	7.40	7.86	0.35	10.10
	DEC	7.38	8.18	0.40	10.80
	JAN	7.38	7.74	0.20	10.85
	FEB	7.32	8.18	0.25	10.25
1977	MAR	7.40	7.88	0.25	10.25
	APR	7.35	7.80	0.30	10.30

APPENDIX THREE

SURVEY MARCH AND OCTOBER 1975

A) Hydrographic data for 59 stations

i) Temperature: March

ii) Temperature : October

iii) Salinity : March

iv) Salinity : October

v) O_2 concentration : March/October

vi) pH : March/October

b) Benthos: Species amd numbers collected.

Ranks for correlation with substratum and water depth.

a) Temperature : March 1975

	DEPTH m.						
	0	0.5	1.0	1.5	2.0	2.5	m Total depth
A1	8.2						0.1
A2	8.2						0.2
A3	8.1						0.4
A4	8.1						0.3
B1	8.2						0.3
B2	8.4	8.0	7.2				1.2
B3	8.3	8.0	7.2	6.8			1.8
B4	8.3	8.0	7.2	6.8			1.7
B5	8.3	8.0					0.9
B6	8.4						0.1
C1	8.4						0.4
C2	8.4	8.0	7.2				1.2
C3	8.3	8.0	7.3	6.8	6.0		2.1
C4	8.3	8.0	7.3	6.9	6.0		2.1
C5	8.3	8.0	7.2	6.8	6.0		2.2
C6	8.3	8.0	7.2	6.8	6.1		2.3
C7	8.4	8.1					0.9
D1	8.4						0.2
D2	8.3	8.2	7.0				1.4
D3	8.3	7.9	6.9				2.2
D4	8.2	7.9	6.9	6.5	6.0		2.4
D5	8.2	7.9	6.8	6.6	6.0		2.8
D6	8.2	7.8	6.8	6.5	6.1	5.8	2.6
D7	8.3	8.1	6.8	6.5	6.1	5.8	2.4
D8	8.3	8.1	7.0	6.5	6.1		1.0
E1	8.3	7.9	7.0				1.1
E2	8.3	7.9	7.0	6.2	5.8		2.0
E3	8.3	7.9	7.0	6.2	5.9		2.4
E4	8.3	7.9	7.1	6.2			1.8

E5	8.3	7.8	7.0	6.2		1.8
E6	8.3	7.7	7.1	6.2		1.9
E7	8.3	7.7	7.1			1.0
E8	8.4					0.2
F1	8.4					0.1
F2	8.4	7.8	7.1			1.2
F3	8.4	7.8	7.0			1.6
F4	8.3	7.9	7.0	6.0		2.2
F5	8.3	7.9	7.0	6.0	6.1	1.4
F6	8.4	7.8	7.1			1.0
F7	8.4	7.8				0.8
F8	8.4	7.8				0.5
G1	8.5					0.4
G2	8.4	7.9	7.2	6.1		1.5
G3	8.4	7.9	7.2	6.0		1.9
G4	8.4	8.0	7.2	6.0		1.9
G5	8.4	8.0				1.1
G6	8.4					0.4
G7	8.4	8.1				0.5
G8	8.4					0.1
H1	8.5					0.1
H2	8.4	8.1	7.2			1.1
H3	8.4	8.1	7.2	6.4		1.8
H4	8.4	8.1	7.2			1.4
H5	8.4	8.1				0.8
H6	8.4					0.4
J1	8.5					0.1
J2	8.5					0.1
J3	8.4					0.4
J4	8.4					0.1

b) Temperature October 1975

	DEPTH m.						
	0	0.5	1.0	1.5	2.0	2.5	Total depth m
A1	10.2						0.1
A2	10.2						0.2
A3	10.1						0.4
A4	10.0						0.3
B1	10.2						0.3
B2	10.2	9.6	9.2				1.2
B3	10.3	9.7	9.2	8.8			1.8
B4	10.3	9.7	9.2	8.8			1.7
B5	10.3	9.6					0.9
B6	10.3						0.1
C1	10.2						0.4
C2	10.3	9.6	9.2				1.2
C3	10.4	9.6	9.8	8.9	8.6		2.1
C4	10.4	9.7	9.8	8.9	8.7		2.1
C5	10.4	9.7	9.2	8.9	8.7		2.2
C6	10.4	9.6	9.2	9.0	8.8		2.3
C7	10.4	9.7					0.9
D1	10.2						0.2
D2	10.2	9.6	9.0				1.4
D3	10.2	9.6	9.1	9.0	8.7		2.2
D4	10.4	9.7	9.0	8.8	8.7		2.4
D5	10.3	9.7	9.0	8.9	8.8	8.0	2.8
D6	10.4	9.7	9.0	8.8	8.7	8.0	2.6
D7	10.4	9.6	9.0	8.8	8.7		2.4
D8	10.4	9.6	9.0				1.0
E1	10.2	9.7	9.0				1.1
E2	10.2	9.7	9.0	8.9	8.9		2.0
E3	10.3	9.6	9.0	8.9	8.9		2.4
E4	10.4	9.7	9.0	8.9			1.8
E5	10.4	9.7	9.1	8.9			1.8

E6	10.4	9.7	9.1	9.0		1.9
E7	10.5	9.6	9.1			1.0
E8	10.5					0.2
F1	10.2					0.1
F2	10.3	9.5	9.2			1.2
F3	10.3	9.6	9.2	9.0		1.6
F4	10.4	9.6	9.2	8.9	8.4	2.2
F5	10.4	9.6	9.2			1.4
F6	10.5	9.5	9.1			1.0
F7	10.5	9.5				0.8
F8	10.6	9.6				0.5
G1	10.3					0.4
G2	10.3	9.7	9.2	9.0		1.5
G3	10.4	9.7	9.2	8.9		1.9
G4	10.4	9.6	9.3	8.9		1.9
G5	10.4	9.7				1.1
G6	10.4					0.4
G7	10.5	9.7				0.5
G8	10.5					0.1
H1	10.3					0.1
H2	10.3	9.6	9.4			1.1
H3	10.4	9.6	9.4	9.1		1.8
H4	10.4	9.7	9.4			1.4
H5	10.5	9.7				0.8
H6	10.5					0.4
J1	10.4					0.1
J2	10.4					0.1
J3	10.4					0.4
J4	10.6					0.1

c) Salinity ‰ March 1975

Station	DEPTH m 0	0.5	1.0	1.5	2.0	2.5	total depth m
A1	17.0						0.1
A2	17.5						0.2
A3	17.5						0.4
A4	17.5						0.3
B1	17.5						0.3
B2	17.5	19.0	20.5				1.2
B3	17.5	19.0	20.5	21.5			1.8
B4	18.0	19.5	21.0	21.5			1.7
B5	18.0	19.5					0.9
B6	18.0						0.1
C1	18.0						0.4
C2	18.0	19.5	21.0				1.2
C3	18.0	19.5	21.0	21.5	22.0		2.1
C4	19.0	20.0	21.0	22.0	22.5		2.1
C5	20.0	20.5	21.0	22.0	22.5		2.2
C6	20.5	20.5	21.0	22.0	22.5		2.3
C7	20.0	20.5					0.9
D1	18.5						0.2
D2	19.0	20.0	21.00				1.4
D3	19.5	20.5	21.0	21.5	22.0		2.2
D4	20.0	20.5	21.0	22.0	22.5		2.4
D5	20.0	20.5	21.0	22.0	22.5	24.0	2.8
D6	20.5	20.5	21.0	22.0	22.5	23.5	2.6
D7	20.5	20.5	20.5	22.0	22.5		2.4
D8	20.0	20.0	20.5				1.0
E1	19.0	20.0	20.5				1.1
E2	19.0	20.0	20.5	22.0	23.0		2.0
E3	19.5	20.5	21.0	22.0	23.0		2.4
E4	19.5	20.5	21.0	22.0			1.8

E5	19.0	20.5				1.8
E6	19.0	20.5				1.9
E7	18.5	20.0				1.0
E8	18.5	19.0				0.2
F1	18.5	18.5				0.1
F2	18.5	19.5	20.5			1.2
F3	19.0	19.5	21.0	22.0		1.6
F4	19.5	20.5	21.0	22.0	23.0	2.2
F5	19.5	20.0	21.0			1.4
F6	19.5	20.0	21.0			1.0
F7	19.0	19.5				0.8
F8	19.0	19.5				0.5
G1	18.5					0.4
G2	19.0	19.5	20.5	21.5		1.5
G3	19.0	19.5	20.5	21.5		1.9
G4	19.5	20.5	21.0	22.0		1.9
G5	19.5	20.5	21.5			1.1
G6	19.5					0.4
G7	19.5	19.5				0.5
G8	19.0	19.0				0.1
H1	19.0	19.0				0.1
H2	19.0	19.5	20.0			1.1
H3	19.0	19.5	21.0	21.5		1.8
H4	19.0	20.0	21.0			1.4
H5	19.0	20.0				0.8
H6	18.5	18.5				0.4
J1	18.0	18.0				0.1
J2	18.0	18.0				0.1
J3	18.0	18.0				0.4
J4	18.5	18.5				0.1

d) Salinity ‰ October 1975

Station	DEPTH m. 0	0.5	1.0	1.5	2.0	2.5	Total depth m
A1	21.0						0.1
A2	21.0						0.2
A3	20.5						0.4
A4	20.0						0.3
B1	18.0						0.3
B2	22.0	22.0	22.0				1.2
B3	22.0	22.0	22.0	22.0			1.8
B4	22.0	22.0	22.0	22.0			1.7
B5	21.5	21.5					0.9
B6	19.0						0.1
C1	18.5						0.4
C2	20.0	22.0	22.0				1.2
C3	22.0	22.0	22.0	22.0	23.0		2.1
C4	22.0	22.0	22.0	22.0	23.0		2.1
C5	22.0	22.0	22.0	22.0	23.0		2.2
C6	22.0	22.0	22.0	22.0	23.0		2.3
C7	19.5	19.5					0.9
D1	18.0						0.2
D2	21.0	21.0	21.5				1.4
D3	21.5	21.5	22.0	22.0	22.0		2.2
D4	21.5	22.0	22.0	22.0	22.0		2.4
D5	22.0	22.0	22.0	22.0	23.0	22.5	2.8
D6	21.5	22.0	22.0	22.0	22.0	22.0	2.6
D7	21.5	22.0	22.0	22.0	22.0		2.4
D8	18.0	20.0					1.0
E1	20.5	21.5					1.1
E2	22.0	22.0	22.0	22.0	22.0		2.0
E3	22.0	22.0	22.0	22.0	22.0		2.4
E4	22.0	22.0	22.0	22.0			1.8

E5	22.0	22.0	22.0	22.0	1.8
E6	22.0	22.0	22.0	22.0	1.9
E7	21.5	21.5	22.0		1.0
E8	18.5				0.2
F1	19.0				0.1
F2	22.0	22.0	22.0		1.2
F3	22.0	22.0	22.0	22.0	1.6
F4	22.0	22.0	22.0	22.0	22.0 2.2
F5	22.0	22.0	22.0		1.4
F6	22.0	22.0	22.0		1.0
F7	22.0	22.0			0.8
F8	21.0	21.5			0.5
G1	18.5				0.4
G2	21.5	22.0	22.0	22.0	1.5
G3	22.0	22.0	22.0	22.0	1.9
G4	22.0	22.0	22.0	22.0	1.9
G5	22.0	22.0	22.0		1.1
G6	22.0				0.4
G7	22.0	22.0			0.5
G8	21.0				0.1
H1	18.5				0.1
H2	22.0	22.0	22.0		1.1
H3	22.0	22.0	22.0	22.0	1.8
H4	22.0	22.0	22.0		1.4
H5	22.0	22.0			0.8
H6	19.0				0.4
J1	18.0				0.1
J2	18.0				0.1
J3	18.0				0.4
J4	18.0				0.1

e) Oxygen March 1975 / October 1975

8 stations monitored only

Station	DEPTH m. 0	0.5	1.0	1.5	2.0	2.5	Total depth m
March							
B1	11.5						0.3
C2	11.5	9.0					1.2
D3	11.5	11.0	9.0	8.5	6.5		2.2
E3	11.5	10.5	10.0	7.5	6.0		2.4
F4	11.5	10.5	9.0	8.0	7.5		2.2
G4	11.5	11.0	10.5	7.0			1.9
H4	11.5	11.0	9.5				1.4
J3	11.5						0.4
October							
B1	11.0						0.3
C2	11.0	11.0	11.0				1.2
D3	11.0	11.0	10.5	8.0	8.0		2.2
E3	11.0	10.5	10.5	9.5	7.5		2.4
F4	11.0	11.0	10.0	9.0	7.5		2.2
G4	11.0	10.5	10.0	8.0			1.9
H4	11.0	10.5	11.0				1.4
J3	11.0						0.4

f) pH March / October 1975

8 stations monitored only

	DEPTH m.						
Station	0	0.5	1.0	1.5	2.0	2.5	Total depth
March							m
B1	7.7						0.3
C2	7.7	7.5					1.2
D3	7.7	7.6	7.4	7.4			2.2
E3	8.1	7.7	7.5	7.4			2.4
F4	8.4	7.7	7.4	7.4			2.2
G4	8.3	7.7	7.4				1.9
H4	8.3	7.7	7.5				1.4
J3	8.2						0.4
October							
B1	7.8						0.3
C2	7.7	7.5					1.2
D3	7.7	7.5	7.5	7.4			2.2
E3	7.8	7.4	7.4	7.4			2.4
F4	7.9	7.5	7.5	7.5			2.2
G4	7.8	7.6	7.5				1.9
H4	7.9	7.6	7.5				1.4
J3	7.9						0.4

b) BENTHOS : SPECIES and NUMBERS COLLECTED

Site	Substratum particle size correlation (ranks 1-13)	Water depth correlation (ranks 1-6)	Sample	Nereis diversicolor.	Capitella capitata.	Arenicola marina.	Corophium volutator.	Littorina rudis.	Hydrobia ulvae.	Cerastoderma glaucum
A1	1	1	A				4	14	3	
			B				1	8	4	..
A2	3	1	A				2	21	3	
			B				2	12	6	
A3	3	1	A				4	6	3	
			B				2	2	0	
A4	10	1	A				1	17		
			B				1	11		
B1	1	1	A	2			2	4	7	
			B	2			1	6	2	
B2	2	3	A				2	9	2	
			B					3		
B3	7	4	A		2			4	2	
			B					2	1	
B4	7	4	A		1		1	9		
			B		2				1	
B5	10	2	A			1				
			B		1				4	
C1	2	1	A	2		3	7	2		
			B			5	11	1		
C2	2	3	A			2		3		
			B					1		
C3	3	5	A			2	3			
			B			1		1		
C4	10	5	A					1		
			B					2		
C5	9	5	A	1						
			B							
C6	10	5	A			2				
			B	1		2	1			
C7	13	2	A			2		1		
			B	5		1	4			

Site	Substratum particle size correlation (ranks 1-13)	Water depth correlation (ranks 1-6)	Sample	Nereis diversicolor.	Capitella capitata.	Arenicola marina.	Corophium volutator.	Littorina rudis.	Hydrobia ulvae.	Cerastoderma glaucum.
D1	1	1	A				4		1	
			B			1	2	3	1	
D2	3	2	A			1		1		
			B				2		1	
D3	10	5	A	1					1	
			B						2	
D4	9	5	A	1					2	
			B				1		4	
D5	10	6	A	1						
			B							
D6	9	6	A	4			1			
			B	1						
D7	9	5	A					1		
			B	2						
D8	13	2	A				4	3	3	
			B	2			1	2	3	
E1	3	3	A						6	3
			B	2		1	4		8	2
E2	2	4	A							6
			B			1				4
E3	10	5	A			1				
			B	2						
E4	10	4	A	1						
			B							
E5	10	4	A	1						
			B	1						
E6	9	4	A	1						1
			B							
E7	9	2	A	1				4		
			B					1		
E8	13	1	A	1				3	4	
			B				3	2	3	

Site	Substratum particle size correlation (ranks 1-13)	Water depth correlation (ranks 1-6)	Sample	Nereis diversicolor.	Capitella capitata.	Arenicola marina.	Corophium volutator.	Littorina rudis.	Hydrobia ulvae.	Cerastoderma glaucum.
F2	3	3	A						2	6
			B			1			9	4
F3	7	4	A		3	1			3	2
			B			1			1	
F4	7	5	A		1	1				
			B							
F5	2	3	A			1				8
			B							2
F6	3	2	A							2
			B			1				1
F7	3	2	A		2		2			
			B							
F8	10	1	A						1	
			B		2		1	2	4	
G1	3	1	A		2			1	4	
			B			1		2	2	
G2	7	3	A		1		1			4
			B			1			2	4
G3	7	3	A			1				
			B			2	1			3
G4	3	3	A							2
			B							3
G5	3	3	A							3
			B							2
G6	10	1	A				1			
			B				1	2		
G7	9	1	A		1			1	6	
			B				4	1	4	
G8	9	1	A		1				2	
			B					2	14	

Site	Substratum particle size correlation (ranks 1-13)	Water depth correlation (ranks 1-6)	Sample	*Nereis diversicolor*	*Capitella capitata*	*Arenicola marina*	*Corophium volutator*	*Littorina rudis*	*Hydrobia ulvae*	*Cerastoderma glaucum*
H1	1	1	A							
			B							
H2	7	3	A	2						1
			B			1			6	4
H3	7	4	A						2	2
			B		1					4
H4	3	3	A	2				1		4
			B			1		2		
H5	3	2	A							1
			B	2						1
H6	13	1	A			1				
			B	1		1	2	1		
J1	10	1	A					3	4	
			B					2	2	
J2	10	1	A					1	14	
			B	1		1		1	9	
J3	10	1	A				1	5	7	
			B	1			3	7	6	
J4	13	1	A	1				1	3	
			B				1		1	

APPENDIX FOUR

Zooplankton Population estimates:

a) Pump sample October 1976 and calculation of net factor:

b) Net Samples 1974 - 1977

i <u>Acartia clausi</u>

ii <u>Eurytemora velox</u>

iii <u>Mesochra lilljeborgi</u>

iv <u>Cerastoderma glaucum</u>

PUMP SAMPLE 15.10.76

Two samples were collected from the centre of the pond at 0.1 m depth using an Allwieler battery operated rotary pump (No.2.). Two 30 l samples were processed using No.8. mesh filters. The samples were treated with pril-formalin (3%) and returned to the laboratory for subsampling. The plankton was transferred to a shaking vessel (round bottomed flask) and the volume made up to 250 ml. Using a stempel pipette, 10 subsamples, each of 1 ml were removed and counted.

The results were as follows:-

Sample I

Acartia clausi	387.23 ± 14.7 /l
Eurytemora velox	100.60 ± 14.9 / l

Sample II

Acartia clausi	378.3 ± 9.4 /l
Eurytemora velox	96.27 ± 11.40 /l

Mean population for A.clausi = $3.83 \times 10^5 / m^3$

Mean population for E. velox = $9.84 \times 10^3 / m^3$

Netted populations collected on the same day gave population estimates

for A. clausi = $2.22 \times 10^5 / m^3$

for E. velox = $6.3 \times 10^3 / m^3$

The efficiency of capture (net factor) for A clausi was calculated as 58% and for E. velox as 64% .

The following data are used to give populations based on a net factor of 60%.

NET SAMPLES 1974 - 1977

Method

Collection: A square net of dimensions 0.33 x 0.33 m^3 (No.8. mesh) was towed at 0.3 m sec^{-1} twice across the pond in a NW - SW direction.

Total distance = 135 m.
Total volume swept = 15 m^3.

Processing: The sample was treated with pril-formalin (3%) and diluted to a litre in a shaking vessel (round-bottomed flask). Using a stempel pipette, 3 subsamples each of 1 ml were removed. Each was diluted fifteen times and a further 1 ml sub-sample taken and counted.

Acartia clausi

YEAR	MONTH	a MEAN SUB-SAMPLE NOS.	b NETTED POPULATION Nos/m^3	c ESTIMATED POPULATION WITH NET FACTOR INCLUDED Nos/m^3
	APR	540	5.40×10^5	9.0×10^5
	MAY	368	3.68×10^5	6.1×10^5
	JUN	348	3.48×10^5	5.8×10^5
1975	JUL	319	3.19×10^5	5.3×10^5
	AUG	194	1.94×10^5	3.2×10^5
	SEPT	416	4.16×10^5	6.9×10^5
	OCT	340	3.40×10^5	5.7×10^5
	NOV	64	0.64×10^5	1.1×10^5
	DEC	1.34	1.34×10^3	2.2×10^3
	JAN	0.34	0.34×10^2	3.7×10^2
	FEB	0.34	0.34×10^2	6.2×10^2
	MAR	198	1.98×10^5	3.3×10^5
	APR	482	4.82×10^5	8.0×10^5
	MAY	484	4.84×10^5	8.1×10^5
1976	JUN	370	3.70×10^5	6.2×10^5
	JUL	355	3.55×10^5	5.9×10^5
	AUG	349	3.49×10^5	5.8×10^5
	SEPT	367	3.68×10^5	6.1×10^5
	OCT	222	2.22×10^5	3.7×10^5
	NOV	2.67	2.67×10^3	4.0×10^3
	DEC	2.67	2.67×10^3	4.2×10^3
	JAN	1.67	1.67×10^3	3.0×10^3
1977	FEB	0.67	0.67×10^3	1.4×10^3
	MAR	1.67	1.67×10^3	2.9×10^3

Eurytemora velox

YEAR	MONTH	a MEAN SUB-SAMPLE Nos.	b NETTED POPULATION Nos/m^3	c ESTIMATED POPULATION WITH NET FACTOR INCLUDED Nos/m^3
	APR	6.67	6.7×10^3	1.1×10^4
	MAY	6.67	6.7×10^3	1.1×10^4
	JUN	6.67	6.7×10^3	1.1×10^4
1975	JUL	5.33	5.3×10^3	8.9×10^3
	AUG	4.00	4.0×10^3	6.7×10^3
	SEPT	10.33	1.03×10^4	1.7×10^4
	OCT	4.67	4.7×10^3	7.8×10^3
	NOV	6.30	6.3×10^3	1.1×10^4
	DEC	5.00	5.0×10^3	8.4×10^3
	JAN	7.00	7.0×10^3	1.2×10^4
	FEB	13.33	1.33×10^4	2.2×10^4
	MAR	4.33	4.3×10^3	7.2×10^3
	APR	2.00	2.0×10^3	3.3×10^3
	MAY	13.33	1.33×10^4	2.2×10^4
1976	JUN	18.67	1.87×10^4	3.1×10^4
	JUL	11.33	1.13×10^4	1.9×10^4
	AUG	5.67	5.67×10^3	9.5×10^3
	SEPT	4.67	4.7×10^3	7.8×10^3
	OCT	6.33	6.3×10^3	1.1×10^4
	NOV	5.33	5.3×10^3	8.9×10^3
	DEC	6.67	6.67×10^3	1.1×10^4
	JAN	6.67	6.67×10^3	1.1×10^4
1977	FEB	4.00	4.0×10^3	6.7×10^3
	MAR	3.00	3.0×10^3	5.0×10^3

Mesochra Lilljeborgi

YEAR	MONTH	a MEAN SUB-SAMPLE Nos.	b NETTED POPULATION Nos/m^3	c ESTIMATED POPULATION WITH NET FACTOR INCLUDED Nos/m^3
	APR			
	MAY			
	JUN	3.67	3.67×10^3	6.1×10^3
1975	JUL	3.0	3.0×10^3	5.0×10^3
	AUG	3.0	3.0×10^3	5.0×10^3
	SEPT			
	OCT			
	NOV			
	DEC			
	JAN			
	FEB			
	MAR	4.0	4.0×10^3	6.7×10^3
	APR	13.66	1.37×10^4	2.28×10^4
	MAY	8.66	8.67×10^3	1.44×10^4
1976	JUN	10.33	1.03×10^4	2.17×10^4
	JUL			
	AUG			
	SEPT			
	OCT			
	NOV			
	DEC			
	JAN			
1977	FEB	0.33	3.3×10^2	5.6×10^2
	MAR	0.33	3.3×10^2	5.6×10^2

Cerastoderma glaucum

YEAR	MONTH	a MEAN SUB-SAMPLE Nos.	b NETTED POPULATION Nos/m^3	c ESTIMATED POPULATION WITH NET FACTOR INCLUDED Nos/m^3
	APR			
	MAY	0·33	$3·30 \times 10^2$	$5·50 \times 10^2$
1975	JUN	1.33	$1·33 \times 10^3$	$2·22 \times 10^3$
	JUL	244.67	$2·45 \times 10^5$	$4·07 \times 10^5$
	AUG	48·00	$0·48 \times 10^5$	$0·80 \times 10^5$
	SEPT			
	OCT			
	NOV			
	DEC			
	JAN			
	FEB			
	MAR	0·67	$0·67 \times 10^3$	$1·11 \times 10^3$
	APR	204·00	$2·04 \times 10^5$	$3·40 \times 10^5$
	MAY	253·33	$2·53 \times 10^5$	$4·22 \times 10^5$
1976	JUN	232·00	$2·32 \times 10^5$	$3·87 \times 10^5$
	JUL			
	AUG			
	SEPT			
	OCT			
	NOV			
	DEC			
	JAN			
1977	FEB			
	MAR			

APPENDIX FIVE

ESTIMATION OF POPULATIONS

OF

Gammarus duebeni

Idotea chelipes

Praunus flexuosus

<u>G. duebeni</u>, <u>I. chelipes</u>: Estimation of populations March/April 1977

<u>Method</u>. A 0.33 x 0.33 m sq. plankton net was adapted to take a metal plate over the aper .ture and placed in a frame so that this could be manipulated on the surface of the pond, in depths up to 0.5 m water. Weed, detritus and pebbles included in the sample were hand sorted for the animals.

A grid system for taking random samples was employed. The area of the pond sampled was limited to that accessible with the technique described, approximately 1.0×10^3 m^2. Forty five samples were taken which approximate 0.5% of the total area available.

Sample	Nos. G. duebeni	Nos. I. chelipes	Sample	Nos. G. duebeni	Nos. I. chelipes
1	0	7	31	5	6
2	4	0	32	6	0
3	7	0	33	9	21
4	3	0	34	4	0
5	0	26	35	0	14
6	0	10	36	10	5
7	11	0	37	0	1
8	0	4	38	3	0
9	9	4	39	0	10
10	0	9	40	4	0
11	14	13	41	0	0
12	0	0	42	12	17
13	22	6	43	5	0
14	0	4	44	0	2
15	4	20	45	2	0
16	5	0			
17	0	8		203	253
18	8	17			
19	0	4			
20	14	3	mean no./sample	4.51	5.62
21	7	0			
22	3	12			
23	6	0			
24	9	1			
25	4	4			
26	0	14			
27	0	0			
28	8	11			
29	3	0			
30	2	0			

G. duebeni	mean no. per sample of 0.11 m^3	= 4.51
	mean no of adults	= 40.5 m^3
	standard error	= ± 9.57
	* area of pond bed	= 5.2×10^3 m^2
	$\hat{N}e$	= $2.10 \pm 0.5 \times 10^5$

* dredging confirmed I. chelipes present at all depths.

I. chelipes	mean no per sample of 0.11 m^3	= 5.62
	mean no of adults	= 50.58 m^3
	standard error	= 8.41
	* area of pond bed 1m in depth	= 2.5×10^3 m^2
	$\hat{N}e$	= $1.26 \pm 0.21 \times 10^5$

* dredging indicated G. duebeni absent in depths gtr. than 1 m.

P. flexuosus

Population estimates based on the Lincoln Index were carried out in April 1977 and March 1979. Data included on page 34.

APPENDIX SIX

CALCULATION AND INTERPRETATION

of

ISOPLETHS

1. an example of the method of calculation
   (Praunus flexuosus : adult : Salts Hole:
   2 mg/l. O_2 conc.)

2. General principles of isopleth interpretation.

Praunus flexuosus ,adult. SALTS HOLE 2mg/l.O_2concentration.

Combined table of replicate experiments:
percentage survival after 120 hours.

	Temperature °C	5		10		15	
	5	0	0	18	6	0	0
salinity ‰	20	50	62	74	70	0	0
	35	18	0	18	20	0	0

Percentages transferred into angular units.(Rohlf,F.J. and Sokal, R., Statistical tables.Ist Ed.,p129.)

Temperature (x_1)	5	10	15 °C
5	0.00	25.10	0.00
	0.00	14.18	0.00
	0.00	39.28	0.00
Salinity (x_2) 20	45.00	59.34	0.00
	51.94	56.79	0.00
	96.94	116.13	0.00
35	25.10	25.10	0.00
	0.00	26.57	0.00
‰	25.10	56.67	0.00

Linear effect for temperature

	5	10	15
	(-1)	(0)	(+1)

at 5‰ = 0.00 - 0.00 = 0.00
at 20‰ = 0.00 -96.96 = -96.94
at 35‰ = 0.00 -25.10 = -25.10

Linear effect for salinity

	5	20	35
	(-1)	(0)	(+1)

at 5°C = 25.10 - 0.00 = 25.10
at 10°C = 56.67 -39.28 = 12.39
at 15°C = 0.00 - 0.00 = 0.00

Quadratic effect for temperature

	5	10	15
	(+1)	(-2)	(+1)

at 5‰ = (39.28 x -2) + 0.00 + 0.00 = -78.56
at 20‰ = (116.13 x -2) + 96.94 + 0.00 = -135.32
at 35‰ = (56.67 x -2) + 25.10 + 0.00 = -78.24

Quadratic effect for salinity

	5	20	35
	(+1)	(-2)	(+1)

at 5°C = (96.94 x -2) + 0.00 + 25.10 = -168.79
at10°C = (116.13 x -2) + 39.28 + 56.67 = -141.33
at15°C = (0.00 x -2) + 0.00 + 0.00 = 0.00

Totalling of effects

For temperature (x_1) at

	5‰	20‰	35‰	Total(Σx_1)
Linear	0.00	-96.94	-25.10	-122.04
Quadratic	-78.79	-135.32	-78.24	-292.12

Tor salinity (x_2) at

	5°C	10°C	15°C	Total(Σx_2)
Linear	25.10	12.39	0.00	37.49
Quadratic	-168.79	-141.33	0.00	-310.09

There now follows the sum of squares expressed over the divisors.
The divisors in the case of a <u>linear</u> plot are expressed as 6(2) = 12.
and in the case of a <u>quadratic</u> plot are expressed as 18(2) = 36.

Sum of squares

Temperature.linear plot = $\frac{(-122.04)^2}{12}$ = 1241.15

Temperature:quadratic plot = $\frac{(-292.12)^2}{36}$ = 2370.39

Salinity: linear plot = $\frac{(37.49)^2}{12}$ = 117.13

Salinity:quadratic plot = $\frac{(-310.09)^2}{36}$ = 2670.99

INTERACTION EFFECTS

Salinity :linear	25.1	12.39	0.00
$S_L \times T_L$	-1	0	+1
$S_L \times T_Q$	+1	-2	+1

$S_L T_L$ = 0.00 -25.1 = -25.1

$S_L T_Q$ = (12.39 x -2) + 25.1 + 0.00 = 00.32

Salinity :quadratic	-168.79	-141.33	0.00
$S_Q \times T_L$	-1	0	+1
$S_Q \times T_Q$	+1	-2	+1

$S_Q T_L$ = 0.00 - 168.79 = 168.79

$S_Q T_Q$ = (-141.33 x -2) -168.79 +0.00 = 113.87

Interaction effects: Sum of squares

	$(\Sigma x)^2$	Divisors	$(\Sigma x)^2$/div.	Result
$S_L T_L$	-25.10	4(2)	$\frac{(-25.10)^2}{8}$	78.75
$S_L T_Q$	0.32	12(2)	$\frac{(0.32)^2}{24}$	0.00
$S_Q T_L$	168.79	12(2)	$\frac{(168.71)^2}{24}$	1186.94
$S_Q T_Q$	113.87	36(2)	$\frac{(113.87)^2}{72}$	180.88

ERROR TERM

This may be expressed as the sum of the squares of each observation minus the correction term.

The correction term is derived as follows :-

$$= \frac{(\text{sum of each observation})^2}{\text{number of observations}}$$

$$\text{Error} = n^2 - \frac{(\Sigma n)^2}{n}$$

Sum of squares of each observation

$$= 25.10^2 + 14.18^2 + \text{etc.....} + 26.57^2$$

$$= \underline{14,266.17} \quad \text{(A)}$$

Sum of each observation

$$= 25.10 + 14.18 + \text{etc.....} + 26.57$$

$$= \underline{329.12}$$

Correction term

$$= \frac{(329.12)^2}{9 \times 2} = = \underline{6,017.78}. \quad \text{(B)}$$

Total variation of the experiment (A-B) = 8248.39

SPECIES: Praunus flexuosus

SITE: Salts Hole O_2 CONC. = 2 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		117.13	2.62
S_Q	1		2670.99	59.78 **
SALINITY	2	2788.12		
T_L	1		1241.15	27.78 **
T_Q	1		2370.39	61.11 **
TEMPERATURE	2	3611.54		
S_LT_L	1		78.75	1.76
S_LT_Q	1		00.00	
S_QT_L	1		1186.94	26.56 **
S_QT_Q	1		180.88	4.05 *
INTERACTION	4	1446.57		
ERROR	9	7846.23	$\frac{8248.39}{-7846.23}$ / 402.16	
TOTAL VARIATION	17	$\frac{402.16}{9}$ =	$\underline{44.68}$	

Error is total variance $V - \frac{\text{total sum of squares}}{\text{degrees of freedom}}$

F ratio is $\frac{\text{means square}}{\text{error}}$

p 0.10 = 3.36 *

p 0.05 = 5.12 **

CALCULATION OF ESTIMATED RESPONSE

$$Y = b_0x_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2 \quad \text{(equation 1)}$$

Now because of the orthogonal design of the experiment

$$\frac{\sum x_1}{n} = \frac{\sum x_2}{n} = \frac{\sum x_1x_2}{n} = 0$$

and also

$$\frac{\sum x_1^2}{n} = \frac{\sum x_2^2}{n} = \frac{2}{3}$$

so in equation 1 using the mean of n's as n_0

$$n_0 = b_0 + \tfrac{2}{3}b_{11} + \tfrac{2}{3}b_{22} \quad \text{(equation 2)}$$

Subtracting equation 2 from 1

$$n = n_0x_0 + b_1x_1 + b_{11}\left(x_1^2 - \tfrac{2}{3}\right) + b_{22}\left(x_2^2 - \tfrac{2}{3}\right) + b_{12}x_1x_2$$

x values may be calculated using more polynomial multiples - thus

Trial	x_o	x_1	x_2	$x_1^2 - \frac{2}{3}$	$x_2^2 - \frac{2}{3}$	x_1x_2	response $\underline{y}$
1	1	-1	-1	$\frac{1}{3}$	$\frac{1}{3}$	1	0.00
2	1	0	-1	$-\frac{2}{3}$	$\frac{1}{3}$	0	20.27
3	1	1	-1	$\frac{1}{3}$	$\frac{1}{3}$	-1	0.00
4	1	-1	0	$\frac{1}{3}$	$-\frac{2}{3}$	0	48.45
5	1	0	0	$-\frac{2}{3}$	$-\frac{2}{3}$	0	58.05
6	1	1	0	$\frac{1}{3}$	$-\frac{2}{3}$	0	0.00
7	1	-1	1	$\frac{1}{3}$	$\frac{1}{3}$	-1	17.45
8	1	0	1	$-\frac{2}{3}$	$\frac{1}{3}$	0	25.84
9	1	1	1	$\frac{1}{3}$	$\frac{1}{3}$	1	0.00
x_2	9	6	6	2	2	4	

y = mean of combined table of replicate experiments(first table of data) in angular units.

$(y)^2 = 20.27^2 + 48.45^2 + \text{etc} \ldots\ldots\ldots 25.84^2$

$= \underline{7,100.63.}$

Calculation of n_0 (1+1+1+1+1+1+1+1+1) = 170.07

Calculation of b_1 (-1+0+1-1+0+1-1+0+1) = -65.91

calculation of b_2 (-1-1-1+0+0+0+1+1+1) = 23.03

calculation of b_{11} ($\frac{1}{3}-\frac{2}{3}+\frac{1}{3}+\frac{1}{3}-\frac{2}{3}+\frac{1}{3}+\frac{1}{3}-\frac{2}{3}+\frac{1}{3}$) = -47.47

calculation of b_{22} ($\frac{1}{3}+\frac{1}{3}+\frac{1}{3}-\frac{2}{3}-\frac{2}{3}-\frac{2}{3}+\frac{1}{3}+\frac{1}{3}+\frac{1}{3}$) = -49.81

calculation of b_{12} (1+0-1+0+0+0-1+0+1) = -17.46

so expressing calculations

Constant	Σx^2	Σyx	$\Sigma yx / \Sigma x^2$	estimate component
n_o	9	170.07	18.90	3213.76
b_1	6	-65.97	-10.99	724.02
b_2	6	23.03	3.84	88.40
b_{11}	2	-47.47	-23.73	1126.66
b_{22}	2	-49.81	-24.91	1240.55
b_{12}	4	-17.46	4.37	76.21

a) TOTAL 6469.60

b) $(y)^2$ 7100.63

Deviation = b) - a) = 631.03

Error variance = $\frac{\text{deviation}}{dF} = \frac{631.03}{3} = 210.34$

$b_0 = 18.90 - \frac{2}{3}b_{11} - \frac{2}{3}b_{22}$ = 51.32 s.e. 8.05

b_1 =-10.99 s.e. 2.72

b_2 = 3.84 s.e. 2.72

b_{11} =-23.73 s.e. 4.73

b_{22} =-24.91 s.e. 4.73

b_{12} = 4.37 s.e. 3.34

S.E.= v

variance v of b = σ^2 / x^2

σ^2 from anovar table = 44.68

so for b_1

s.e. = $\frac{44.68}{6}$ = 2.72

this equation holds except for b_o

v of b_o = 0.5555 x o^2

so S.E. = 4.98

CALCULATION OF RESPONSE SURFACE CENTRE (x_s)

$$\hat{Y} = b_0x_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2$$

$$= 51.32 - 10.99x_1 + 3.84x_2 - 23.73x_1^2 - 24.91x_2^2 + 4.37x_1x_2$$

Differentiating with respect to x_1

$$-2(23.73x_1) + 4.37x_2 = 10.99 \quad \ldots\ldots\ldots\ldots\ldots(1)$$

Differentiating with respect to x_2

$$4.37x_1 - 2(24.91x_2) = -3.84 \quad \ldots\ldots\ldots\ldots\ldots(2)$$

Solving equations (1) and (2)

$$x_{1s} = -0.23$$
$$x_{2s} = +0.06$$

These values are sustituted in the original equation to find the value of $\hat{Y}$.

Optimum yield $\hat{Y} = 52.67$

Transposing from angular units.

Temperature response surface centre = x_{1s} = 8.85°C.

Salinity response surface centre = x_{2s} = 20.90‰

Optimum yield = $\hat{Y}$ = 63.20%

Calculation of roots for plotting isopleths.

Quadratic equations are derived from the original equation.

$$\hat{Y} = 51.32 - 10.99x_1 + 3.84x_2 - 23.73\,x_1^2 - 24.91x_2^2 + 4.37x_1x_2.$$

For values of x_1

$$23.73x_1^2 + (10.99 - 4.37x_2)x_1 + (Y - 51.32 - 3.84x_2 + 24.91x_2^2) = 0$$

For values of x_2

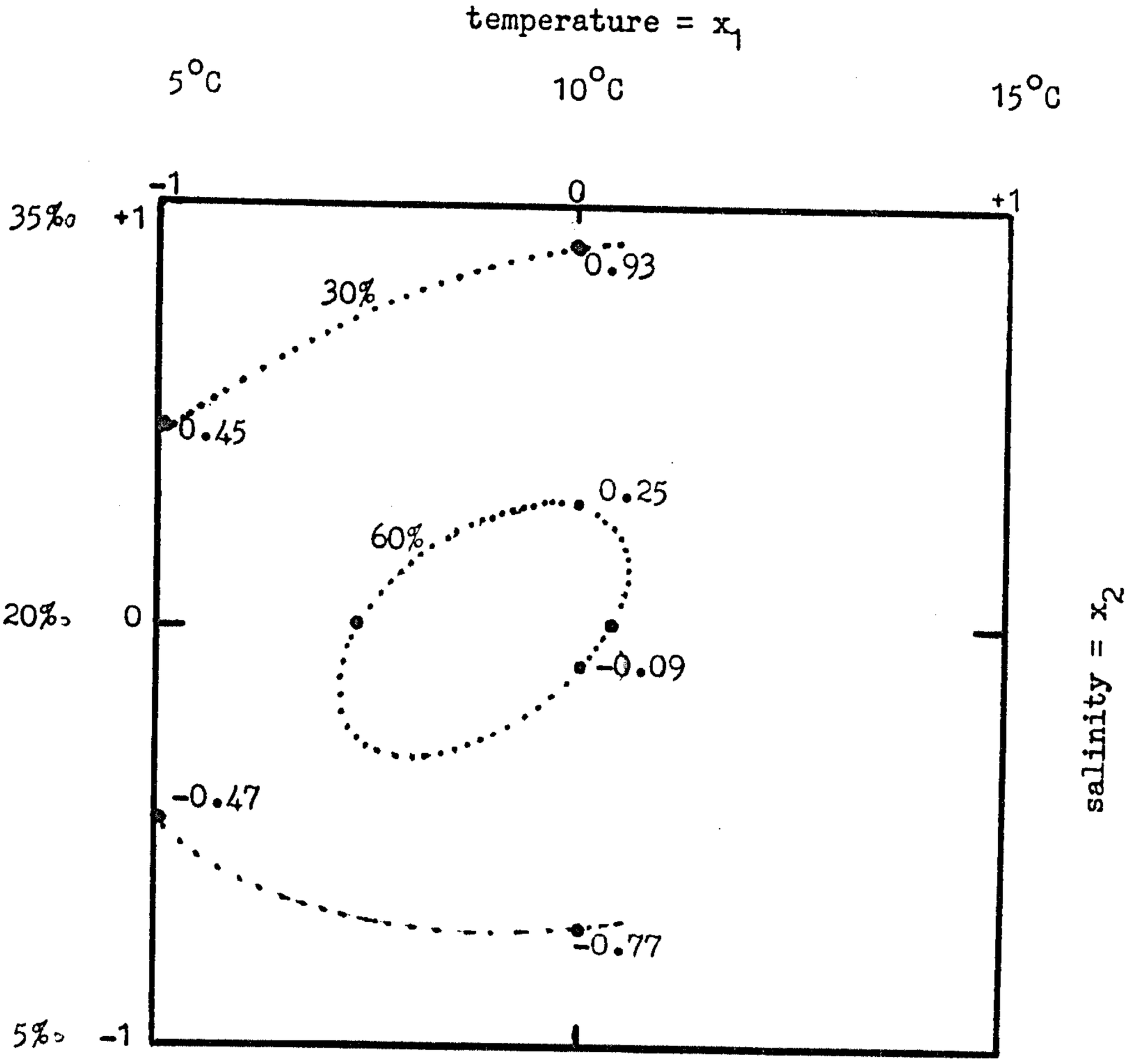
$$24.91x_2^2 + (-3.84 - 4.37x_1)x_2 + (Y - 51.32 + 10.99x_1 + 23.73x_1^2) = 0$$

These equations are then solved eg.

Value of $\hat{Y}$	= angular transn.	values of x when	a	b	c	ROOTS
60	50.77	$x_1 = 0$	24.91	-3.84	-0.57	0.25 -0.09
60	50.77	$x_2 = 0$	23.73	10.99	-0.57	0.05 -0.51
		etc				
30	33.21	$x_1 = 0$	24.91	-3.84	-18.13	0.93 -0.77
30	33.21	$x_1 = -1$	24.91	0.53	-5.37	0.45 -0.47

The roots are plotted .Since the design is orthogonal,the central value for each parameter is zero ,the lower -1 and the upper +1

For example ,plotting the roots above,



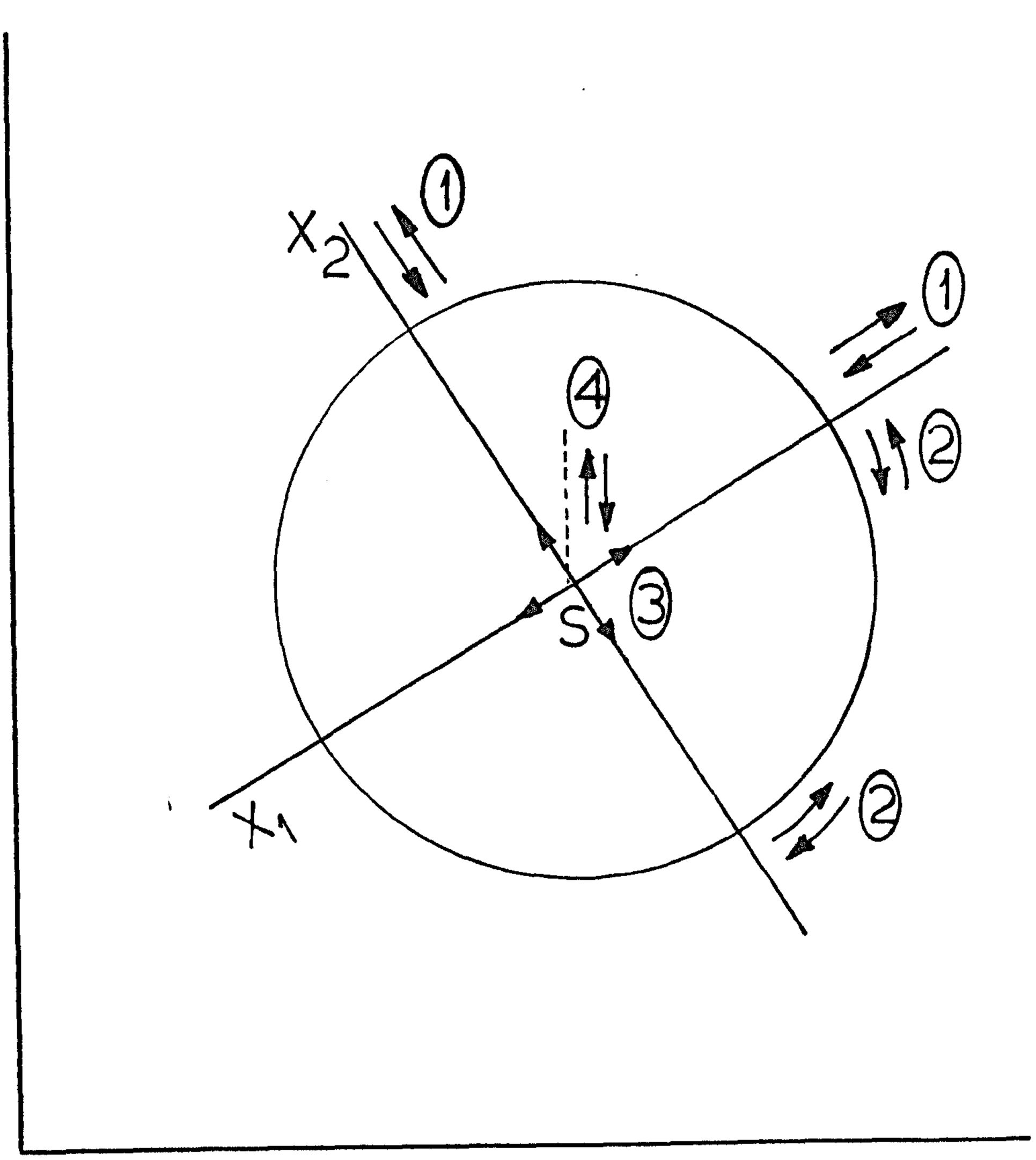
In practice, of course, it is necessary to establish roots at many points in order to determine the form of the contour.

2. BASIC PROPERTIES OF BIOLOGICAL RESPONSE SURFACES

Examination of biological response surfaces in the manner outlined indicates that they are dynamic. Surface configuration and surface location may change with respect to the axes of measurement. These variations may be associated both with genetic and non-genetic differences (i.e., in state of growth, size, physiological condition, previous history, or season). The manner in which such chenges can occur is illustrated in the diagram overleaf. The circular response surface in the figure is analogous to the surface projected on the x_1, x_2 axes of fig. 15 on page 42. The types of surface variation are analogous to four classes of biological phenomena:

(1) Euryplasticity and stenoplasticity. These terms, as usually employed, relate to the ability of an organism to live successfully either within a broad or a narrow range of levels of an environmental variable. A quantitative connotation is implied in their use, yet not without considerable problems of interpretation. The major problem appears in the effort to relate a quantitative measure of viability to a measure of the range of the environmental variable normally available to the organism. The quantitative relationship would then be expressed, somewhat arbitrarily, in a measure of the extent to which the available range is or is not successfully utilized. The relation may be extended to include a comparison of stages of development (egg, larva, juvenile, adult) for a given species. The concept would appear also to force a distinction between limitations on viability within an available range imposed by the organism, as contrasted with restrictions which might be imposed by the available range itself (e.g., deep sea species).

The quantitative aspects of response-surface analysis invite an attempt to 'measure' plasticity. For example, the relative lengths of the X_1 and X_2 axes of a response-surface can vary within and between species for a given set of environmental variables x_1 and x_2. For a euryplastic organism, for instance, the X_1- axis of the surface may stretch over a considerable range of the environmental variable x_1 normally available to the organism. High response values, centered around S, could then extend in the direction of the X_1- axis over a considerable portion of the natural range of x_1. For a stenoplastic organism, the reverse would be true; the length of the X_2- axis could be short with reference to the range of the x_2 variable normally available to the organism.



DYNAMIC PROPERTIES OF RESPONSE SURFACES.

(1) CHANGES IN THE LENGTHS OF THE X AXES (PLASTICITY).
(2) ROTATION OF THE X AXES ABOUT THE CENTRE,S (INTERACTIONS).
(3) TRANSLATION OF THE CENTRE OF THE SURFACE (CHANGES IN TOLERANCE OR RESISTANCE).
(4) CHANGES IN MAGNITUDE OF RESPONSE AT THE CENTRE,S (CHANGES IN CAPACITY).

Thus, the region of high response values around the centre S and in the direction of the X_2- axis could be limited with respect to the natural range of x_2.

The argument will not be forced.Nevertheless,as an example, an organism might be considered euryplastic in terms of a given response if 90% or more of maximum response (e.g. survival) were possible over 50% of the normally available range of the environmental variable considered.

(2) Interaction effects. The principal axes of the surface may rotate about the centre of the surface S.That is,the maximum response obtainable at a given level of one variable will depend on the level of another variable simultaneously applied. Rotation of the response surface axes therefore,indicates the presence of a coupled action, or interaction of environmental variables.The coupled relationship may be calculated for a surface equation by entering a selected value of one variable (e.g. x_1) into the equation,taking the derivative of the fitted equation with respect to the second variable (x_2),setting the equation to zero and solving for x_2.

(3) Changes in tolerance or resistance. The location of the centre of a response surface S,may change (e.g. with time) in regard to the x_1,x_2 scales of measurement.With growth and development of the organism, maximum response may shift to another locus of x_1 and x_2. Hence,as the surface changes location,response scores obtained on a specific pathway in the experimental space may change (e.g. over several levels of x_2 at a constant level of x_1). Therefore,changes in resistance or tolerance would be noted as changes in response scores with respect to the axes of measurement over selected transects of compared surfaces.

(4) Changes in capacity. With growth and development of an organism, the absolute value of the response at S may increase or decrease. Conversely,a change in tolerance or resistance could occur,resulting from a change in location of a response surface,without a change necessarily occurring in the capacity of the surface. Therefore,changes in capacity would be noted by changes in absolute magnitude of response at the centres of compared surfaces.

APPENDIX SEVEN

ESTABLISHMENT OF PARAMETERS FOR CRUSTACEAN SPECIES

a) Preliminary experiments

b) Kruskal-Wallis tests.

Salinity	M	F	M+F			M	F	M+F			M	F	M+F		
‰	a	b	c	$\bar{x}$	S	a	b	c	$\bar{x}$	S	a	b	c	$\bar{x}$	S
5	7	3	6	5.334	1.699	5	3	5	4.334	0.942	3	1	1	1.667	0.942
10	38	34	34	35.334	1.886	37	34	31	34.000	2.449	30	32	27	29.667	2.054
15	35	37	33	35.000	1.633	33	36	32	33.667	1.700	32	28	27	29.000	2.160
20	48	48	49	48.334	0.473	47	48	48	47.667	0.471	40	43	38	40.334	2.054
25	48	50	50	49.334	0.943	48	49	48	48.667	0.471	44	44	40	42.667	1.886
30	50	47	50	49.000	1.414	50	46	47	47.667	1.700	42	42	39	41.000	1.414
35	46	50	48	48.000	1.633	45	48	48	47.000	1.414	41	43	36	40.000	2.944
40	28	17	19	21.000	4.769	19	17	14	16.667	2.053	6	11	11	9.334	2.356
45	29	18	14	20.334	6.342	22	17	12	17.000	4.082	11	6	9	8.667	2.054
	24 hours		1			48 hours		2			72 hours		3		

Idotea chelipes

Determination of Parameters 1) Salinity numbers surviving

a) 50 Males b) 50 Females c) 25 Males and 25 Females

Salinity ‰	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s
5	3	1	1	1.667	0.943	2	1	0	1	1.000	0	0	0	-	-
10	30	31	26	29.000	2.160	30	27	26	27.667	2.888	30	22	26	.260	3.266
15	31	27	25	27.667	2.082	29	27	24	26.667	2.055	29	27	23	26.334	2.495
20	39	42	38	39.667	1.700	39	40	36	38.334	1.700	38	40	36	38.000	1.632
25	43	44	40	42.334	1.700	43	44	38	41.667	2.625	43	41	38	40.667	2.055
30	40	39	33	37.334	3.091	40	39	32	37.000	3.560	38	39	32	36.334	3.091
35	33	39	31	34.33	3.400	27	38	26	30.334	5.436	22	30	27	26.333	3.367
40	3	7	3	4.333	1.885	0	4	1	1.667	1.401	0	2	1	1	1.000
45	4	0	0	4.000	-	0	0	0	-	-	0	0	0	-	-
	96 hours			4		120 hours			5		144 hours			6 days	

Idotea chelipes

Determination of Parameters 1) Salinity numbers surviving

a) 50 males b) 50 females c) 25 males and 25 females

mg/l	O_2	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s
8		50	48	50	49.334	0.943	48	48	48	48	0.000	46	48	47	47	0.816
6		48	49	50	49.000	0.816	47	47	50	48	1.414	46	47	49	47.333	1.247
4		48	50	46	48.000	1.633	47	48	45	46.667	1.247	47	46	45	46	0.816
2		33	38	40	37.000	2.944	32	31	27	30	2.160	29	17	11	19	7.483

24 hours 1 48 hours 2 72 hours 3

Idotea chelipes

Determination of parameters 2) O_2 saturation numbers surviving

a) 50 males b) 50 females c) 25 males and 25 females

mg/l O_2	96 hours 4					120 hours 5					144 hours 6				
	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s
8	43	45	45	44.334	0.943	41	44	45	43.334	1.700	40	44	43	42.334	1.700
6	45	47	49	47	1.633	43	45	48	45.334	2.054	41	42	44	42.334	1.247
4	45	46	44	45	1.633	44	43	43	42.334	1.700	41	41	36	39.334	2.357
2	16	12	9	12.334	2.867	15	11	8	11.334	2.867	15	11	8	11.334	2.867

Idotea chelipes

Determination of parameters 2) O_2 concentration numbers surviving

a) 50 males b) 50 females c) 25 males and 25 females

Salinity ‰	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s
5	62	56	52	56.67	4.11	56	50	38	48	7.48	42	44	28	38	7.12
10	72	62	60	64.67	5.25	64	60	52	58.67	4.99	60	58	50	56	4.32
15	100	100	100	100	0.00	100	90	100	96.67	4.71	92	86	100	92.67	5.74
20	96	100	94	96.67	2.49	96	100	94	96.67	2.49	92	96	90	92.67	2.49
25	98	96	98	97.33	0.94	98	90	98	95.33	3.77	98	90	96	94.67	3.40
30	78	90	90	86	5.73	72	82	74	76.00	4.32	60	76	64	66.67	6.80
35	62	80	64	68.67	12.18	56	68	48	57.33	8.22	52	60	44	51.00	6.53
40	42	22	60	41.33	15.52	30	12	14	18.67	8.06	12	2	2	5.33	4.71

24 hours 48 hours 72 hours

Praunus flexuosus

Determination of salinity parameters % survival

a) 50 males b) 50 females c) 25 males and 25 females

Salinity ‰	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s
	96 hours					120 hours					144 hours				
5	38	40	12	30	12.75	28	36	6	23.33	12.84	14	30	0	14.67	12.26
10	58	58	48	54.67	4.71	54	58	40	50.67	7.51	54	58	36	49.33	9.57
15	90	84	100	91.33	5.01	88	84	94	88.67	4.11	88	80	92	86.67	4.99
20	92	94	86	90.67	3.40	92	90	80	87.33	5.25	92	90	80	87.33	5.25
25	96	88	94	92.67	3.40	96	86	90	90.67	4.11	96	84	88	89.33	4.99
30	56	68	60	61.33	4.99	54	68	56	59.33	6.18	50	64	54	56.00	5.89
35	46	54	40	46.67	5.73	40	48	32	40	6.53	36	40	30	35.33	4.11
40	0	0	0	-	-	0	0	0	-	-	0	0	0	-	-

Praunus flexuosus

Determination of salinity parameters % surviving

a) 50 males b) 50 females c) 25 males and 25 females

Salinity ‰	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s
				24 hours					48 hours					72 hours	
5	94	90	84	89.33	4.11	90	88	80	86	4.98	82	84	76	80.67	3.39
10	90	88	84	87.33	2.49	86	88	80	84.67	3.39	82	88	78	82.67	4.11
15	92	100	98	96.67	3.39	92	100	98	96.67	3.39	90	98	98	95.33	3.77
20	92	98	100	96.67	3.39	92	96	100	96	3.27	88	94	100	94.00	4.90
25	100	100	94	98.00	1.63	98	100	94	97.33	2.49	98	98	92	96	2.83
30	94	92	100	95.33	3.39	94	92	90	92	1.63	94	92	88	91.33	2.49
35	94	90	100	94.67	4.11	92	88	92	90.67	1.89	90	80	88	86	4.32
40	88	94	92	91.33	2.49	80	94	90	88	5.89	78	86	86	83.33	3.77

Gammarus duebeni

Determination of Salinity parameters % surviving

a) 50 males b) 50 females c) 25 males and 25 females

Salinity ‰	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s	a	b	c	$\bar{x}$	s
	96 hours					120 hours					144 hours				
5	76	78	72	75.33	2.49	74	70	70	71.33	1.89	70	68	68	68.67	0.94
10	80	86	74	80.00	4.89	80	86	74	80	4.89	78	84	74	78.67	4.22
15	90	96	96	94	2.83	90	92	96	92.67	2.49	90	90	96	92	2.83
20	88	92	94	91.33	2.49	88	92	90	90	1.63	86	92	90	89.33	2.49
25	96	98	92	95.33	2.49	96	94	92	94	1.63	96	92	92	93.33	3.13
30	90	82	82	84.66	3.77	86	80	74	80	4.90	80	76	74	76.67	2.49
35	84	76	84	81.33	3.69	80	74	84	79.33	4.11	80	70	82	77.33	5.25
40	72	80	82	78	4.32	68	78	78	74.66	4.71	68	74	70	70.66	2.49

Gammarus duebeni

Determination of salinity parameters % surviving

a) 50 males b) 50 females c) 25 males and 25 females

KW TEST ON 72 HOUR SAMPLE Idotea chelipes Salinity.

BLOCK treatments		a	b	c
5	i	3	1	1
10	ii	30	32	27
15	iii	32	28	27
‰ 20	iv	40	43	38
25	v	44	44	40
30	vi	42	42	39
35	vii	41	43	36
40	viii	6	11	11
45	ix	11	6	9

observations

	a	b	c
i	3	1½	1½
ii	13	14½	10½
iii	14½	12	10½
iv	19½	24½	17
v	26½	26½	19½
vi	22½	22½	18
vii	21	24½	16
viii	4½	8	8
ix	8	4½	6

ranks

Ri	132.5	138.5	107	Ri	378
Ri^2	17556.25	19182.25	11449	Ri^2	27024.432
ni	9	9	9	ni	27
Ri^2/ni	1950.69	2131.36	1272.11	Ri^2/ni	5354.1666

$$H = \frac{12}{n(n+1)} - 3(n+1)$$

$$= \frac{12}{27\ (28)} \quad 5354.1666 \quad -3\ (28)$$

$$= 0.01587 \times 5354.1666 - 84$$

$$= 84.9867 - 84$$

$$H = \underline{0.9867}.$$

entitled with the conventional degree of confidence to accept the null hypothesis of the test that is to believe that the medians of the three populations are similar.

TEST ON 72 HOUR SAMPLE Idotea chelipes O_2 CONCENTRATION

BLOCK					
Treat-ments		a	b	c	
	i	46	48	47	
	ii	46	47	49	observations
	iii	47	46	45	
	iv	29	17	11	
ranks	i	6	11	9	
	ii	6	9	12	
	iii	9	6	4	
	iv	3	2	1	
Ri		24	28	25	Ri 78
Ri^2		576	784	675	
ni		4	4	4	ni 12
Ri^2/ni		144	196	169	Ri^2/ni 509

$$H = \frac{12}{n\ (n+1} - 3\ (n+1)$$

$$= \frac{12}{12\ (13)} \quad 509 \quad - \ 3\ (12+1)$$

$$= \quad 39.153 \quad - \ 39 \quad = \ \underline{0.153}$$

entitled with the conventional degree of confidence to accept the null hypothesis of the test that is to believe that the medians of the three populations are similar.

KW TEST ON 120 HOUR SAMPLE Gammarus duebeni SALINITY

BLOCK

Treat-ments	a	b	c
i	74	70	70
ii	80	86	74
iii	90	92	90
iv	88	92	90
v	96	94	92
vi	86	80	74
vii	80	74	84
viii	68	78	78

observations

i	$5\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{2}$
ii	11	$14\frac{1}{2}$	$5\frac{1}{2}$
iii	$17\frac{1}{2}$	20	$23\frac{1}{2}$
iv	16	20	$17\frac{1}{2}$
v	$23\frac{1}{2}$	22	20
vi	$14\frac{1}{2}$	11	$5\frac{1}{2}$
vii	11	$5\frac{1}{2}$	13
viii	1	$8\frac{1}{2}$	$8\frac{1}{2}$

Ranks

Ri	100	104	96	Ri 300
Ri^2	10,000	10816	9216	Ri^2 30,032
ni	8	8	8	ni 24
Ri^2/ni	1250	1352	1152	Ri^2/ni 3754.00

$$H = \frac{12}{n(n+1)} - 3(n+1)$$

$$= \frac{12}{24(25)} \quad 3740.0 - 3 \times 25$$

$$= 75.09 - 75$$

$$= \underline{0.09}$$

entitled with conventional degree of confidence to accept the null hypothesis of the test, i.e. that the medians of the 3 populations are similar.

KW TEST ON 120 HOUR SAMPLE Praunus flexuosus SALINITY

BLOCK

	a	b	c	
i	28	36	6	
ii	54	58	40	
iii	88	84	94	
iv	92	90	80	
v	96	86	90	Observations
vi	54	68	56	
vii	40	48	32	
viii	0	0	0	

i	2	4	1	
ii	$8\frac{1}{2}$	11	$5\frac{1}{2}$	
iii	16	14	20	
iv	19	$17\frac{1}{2}$	13	
v	21	15	$17\frac{1}{2}$	Ranks
vi	$8\frac{1}{2}$	12	10	
vii	$5\frac{1}{2}$	7	3	
viii	-	-	-	

Ri	80.5	80.5	70	
Ri^2	6480.25	6480.25	4900	
ni	7	7	7	ni = 21
Ri^2/ni	925.75	925.75	700	Ri^2/ni = 2551.5

$$H = \frac{12}{n(n+1)} - 3(n+1)$$

$$= \frac{12}{21(22)} \quad 2551.5 \quad - \quad 66$$

$$= (0.026 \times 2551.5) \quad - \quad 66$$

$$= 66.34$$

$$= 0.34$$

=====

entitled with conventional degree of confidence to accept the null hypothesis of the test.i.e. that the medians of the three populations are similar

APPENDIX EIGHT

ANOVAR TABLES FOR ALL CRUSTACEAN SAMPLES.

A). Idotea chelipes , adults
B) Gammarus duebeni, adults
C) Gammarus duebeni, juveniles
D) Praunus flexuosus, adults
E) Praunus flexuosus, juveniles

SPECIES: Idotea chelipes ADULT

SITE: Salts Hole O_2 CONC. = 2 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		1154.64	45.39 **
S_Q	1		2111.25	82.98 **
SALINITY	2	3265.87		
T_L	1		876.03	34.44**
T_Q	1		1353.38	53.19**
TEMPERATURE	2	2229.41		
S_LT_L	1		2.33	0.09
S_LT_Q	1		116.25	4.47*
S_QT_L	1		143.37	5.63**
S_QT_Q	1		901.71	35.44**
INTERACTION	4	1163.66		
ERROR	9	6658.94	6887.93 -6658.94 228.99	
TOTAL VARIATION	17	$\frac{228.99}{9}$ =	25.44	

SPECIES: Idotea chelipes ADULT

SITE: Salts Hole O_2 CONC. = 5 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		1385.89	30.37**
S_Q	1		1457.46	31.94**
SALINITY	2	2843.35		
T_L	1		1217.26	26.28**
T_Q	1		1872.87	41.04**
TEMPERATURE	2	3090.13		
S_LT_L	1		66.24	1.45
S_LT_Q	1		147.21	3.23
S_QT_L	1		672.78	14.72**
S_QT_Q	1		82.15	1.80
INTERACTION	4	968.39		
ERROR	9	6901.87	6901.87 - 6491.23 = 410.64	
TOTAL VARIATION	17		$\frac{410.64}{9}$ = 45.63.	

SPECIES: Idotea chelipes ADULT

SITE: SALTS HOLE O_2 CONC. = 8 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		958.90	60.23**
S_Q	1		278.83	17.51**
SALINITY	2	1237.73		
T_L	1		971.82	61.04**
T_Q	1		533.53	33.51**
TEMPERATURE	2	1505.35		
S_LT_L	1		203.12	12.75**
S_LT_Q	1		100.81	6.33**
S_QT_L	1		262.22	16.47**
S_QT_Q	1		9.63	0.60
INTERACTION	4	575.78		
ERROR	9	3318.86	3462.19 - 3318.86 = 143.33	
TOTAL VARIATION	17	$\frac{143.99}{9}$ = 15.92		

SPECIES: Idotea chelipes ADULT

SITE: Holkham Bay O_2 CONC. = 2 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		5133.79	133.81**
S_Q	1		1159.52	30.23**
SALINITY	2	6291.71		
T_L	1		4549.36	118.59**
T_Q	1		593.98	15.48**
TEMPERATURE	2	5143.34		
S_LT_L	1		305.17	7.96**
S_LT_Q	1		247.75	6.46**
S_QT_L	1		656.47	17.10**
S_QT_Q	1		173.36	4.52*
INTERACTION	4	1382.74		
ERROR	9	12817.79	13163.02 -12817.79 345.23	
TOTAL VARIATION	17	$\frac{345.23}{9}$ = 38.36		

SPECIES: Idotea chelipes ADULT

SITE: HOLKHAM BAY O_2 CONC. = 5 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		5009.02	1123.09 **
S_Q	1		663.49	149.76 **
SALINITY	2	5672.51		
T_L	1		3488.77	782.23 **
T_Q	1		2568.29	575.84 **
TEMPERATURE	2	6057.06		
S_LT_L	1		1093.01	245.07 **
S_LT_Q	1		73.46.	16.47 **
S_QT_L	1		690.05	154.82 **
S_QT_Q	1		114.64	25.70 **
INTERACTION	4	1971.16		
ERROR	9	13700.73	13740.89 − 13700.73 = 40.16	
TOTAL VARIATION	17	$\frac{40.16}{9}$ = 4.46		

SPECIES: Idotea chelipes ADULT

SITE: HOLKHAM BAY O_2 CONC. = 8 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		1481.18	28.59 **
S_Q	1		333.91	6.45 **
SALINITY	2	1515.09		
T_L	1		2518.36	48.62 **
T_Q	1		1908.23	36.84 **
TEMPERATURE	2	4426.59		
S_LT_L	1		120.67	2.33
S_LT_Q	1		7.09	0.14
S_QT_L	1		368.87	7.12 **
S_QT_Q	1		0.67	0.01
INTERACTION	4	497.30		
ERROR	9	6438.98	6905.74 -6438.98 466.76	
TOTAL VARIATION	17		$\frac{466.76}{9}$ = 51.8	

SPECIES: Gammarus duebeni ADULT

SITE: SALTS HOLE O_2 CONC. = 2 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		0.01	0.00
S_Q	1		26.15	1.17
SALINITY	2	26.16		
T_L	1		2.29	0.10
T_Q	1		356.20	15.97 **
TEMPERATURE	2	358.49		
S_LT_L	1		0.86	0.04
S_LT_Q	1		6.91	0.31
S_QT_L	1		0.29	0.01
S_QT_Q	1		21.30	0.95
INTERACTION	4	414.01		
ERROR	9		614.81 -414.01 200.80	
TOTAL VARIATION	17	$\frac{200.80}{9}$ =	22.31	

SPECIES: Gammarus duebeni ADULT

SITE: SALTS HOLE O_2 CONC. = 5 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		66.50	13.88 **
S_Q	1		275.28	57.46 **
SALINITY	2	341.78		
T_L	1		1.85	0.39
T_Q	1		352.38	73.56 **
TEMPERATURE	2	354.22		
S_LT_L	1		68.21	14.24 **
S_LT_Q	1		7.84	1.64
S_QT_L	1		31.04	6.48 **
S_QT_Q	1		155,99	32.57 **
INTERACTION	4	263.08		
ERROR	9	959.08	1002.13 - $\frac{959.08}{43.05}$	
TOTAL VARIATION	17		$\frac{43.05}{9}$ = 4.79	

SPECIES: Gammarus duebeni ADULT

SITE: SALTS HOLE O_2 CONC. = 8 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		115.88	8.25 **
S_Q	1		193.53	13.78 **
SALINITY	2	309.41		
T_L	1		20.38	1.45
T_Q	1		313.88	22.36 **
TEMPERATURE	2	334.26		
S_LT_L	1		21.16	1.51
S_LT_Q	1		35.07	2.50
S_QT_L	1		2.98	0.21
S_QT_Q	1		340.04	24.24 **
INTERACTION	4	399.25		
ERROR	9	1042.93	$1169.77 - \frac{1042.93}{126.34}$	
TOTAL VARIATION	17	$\frac{126.34}{9} = 14.04$		

SPECIES: Gammarus duebeni ADULT

SITE: HOLKHAM BAY O_2 CONC. = 2 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		34.37	3.72 *
S_Q	1		16.20	1.76
SALINITY	2	50.57		
T_L	1		13.42	1.46
T_Q	1		360.43	39.2 **
TEMPERATURE	2	373.85		
S_LT_L	1		3.92	0.42
S_LT_Q	1		3.69	0.40
S_QT_L	1		18.27	1.98
S_QT_Q	1		0.15	0.00
INTERACTION	4	25.93		
ERROR	9	450.36	553.12 -450.36 82.71	
TOTAL VARIATION	17	$\frac{82.71}{9}$ = 9.19		

SPECIES: Gammmarus duebeni Adult

SITE: HOLKHAM BAY O_2 CONC. = 5 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		29.67	1.87
S_Q	1		352.63	22.25 **
SALINITY	2	382.30		
T_L	1		1.64	0.10
T_Q	1		13.80	5.77 **
TEMPERATURE	2	91.10		
S_LT_L	1		6.49	0.41
S_LT_Q	1		13.80	0.87
S_QT_L	1		240.58	15.18 **
S_QT_Q	1		1.82	0.11
INTERACTION	4	262.69		
ERROR	9	736.09	878.71 -736.09 142.62	
TOTAL VARIATION	17	$\frac{142.62}{9}$ =	15.85	

SPECIES: Gammarus duebeni ADULT

SITE: HOLKHAM BAY O_2 CONC. = 8mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		77.11	2.10
S_Q	1		778.04	21.22 **
SALINITY	2	855.15		
T_L	1		4.95	0.13
T_Q	1		15.59	0.43
TEMPERATURE	2	20.54		
S_LT_L	1		40.95	1.11
S_LT_Q	1		0.00	0.00
S_QT_L	1		2.02	0.06
S_QT_Q	1		50.37	1.37
INTERACTION	4	93.34		
ERROR	9	969.03	1299.10 -969.03 330.07	
TOTAL VARIATION	17	$\frac{330.07}{9}$ =	36.67	

SPECIES: Gammarus duebeni - JUVENILE

SITE: SALTS HOLE O_2 CONC. = 2 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		62.24	1.52
S_Q	1		246.23	5.99 **
SALINITY	2	308.47		
T_L	1		2.19	0.05
T_Q	1		359.54	8.75 **
TEMPERATURE	2	361.73		
S_LT_L	1		1.12	0.03
S_LT_Q	1		1.70	0.04
S_QT_L	1		36.53	0.89
S_QT_Q	1		75.22	1.83
INTERACTION	4	114.57		
ERROR	9	784.77	$1154.34 - \frac{784.77}{369.57}$	
TOTAL VARIATION	17	$\frac{369.57}{9}$ =	41.06	

SPECIES: Gammmarus duebeni JUVENILE

SITE: HOLKHAM BAY O_2 CONC. = 2 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		0.32	0.01
S_Q	1		75.95	2.01
SALINITY	2	76.27		
T_L	1		2.21	0.06
T_Q	1		165.76	4.38 *
TEMPERATURE	2	167.97		
S_LT_L	1		10.42	0.28
S_LT_Q	1		0.57	0.02
S_QT_L	1		4.60	0.12
S_QT_Q	1		9.90	0.18
INTERACTION	4	22.49		
ERROR	9	266.73	607.21 -266.73 340.48	
TOTAL VARIATION	17	$\frac{340.48}{9}$ = 37.83		

SPECIES: Praunus flexuosus ADULT

SITE: SALTS HOLE O_2 CONC. = 2 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		117.13	2.62
S_Q	1		2670.99	59.78 **
SALINITY	2	2788.12		
T_L	1		1241.15	27.78 **
T_Q	1		2370.39	61.11 **
TEMPERATURE	2	3611.54		
S_LT_L	1		78.75	1.76
S_LT_Q	1		0.00	0.00
S_QT_L	1		1186.94	26.56 **
S_QT_Q	1		180.88	4.05 *
INTERACTION	4	1446.57		
ERROR	9	7946.23	8248.39 -7846.23 402.16	
TOTAL VARIATION	17	$\frac{402.16}{9}$ = 44.68		

SPECIES: Praunus flexuosus ADULT

SITE: SALTS HOLE O_2 CONC. = 5 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		12.73	0.59
S_Q	1		2801.58	130.94 **
SALINITY	2	2814.31		
T_L	1		4266.51	199.44 **
T_Q	1		1394.65	65.17 **
TEMPERATURE	2	5661.16		
S_LT_L	1		8.56	0.40
S_LT_Q	1		0.00	0.00
S_QT_L	1		21.19	0.99
S_QT_Q	1		158.15	7.37 **
INTERACTION	4	187.90		
ERROR	9	8663.37	8855.87 $-\underline{8663.37}$ 192.50	
TOTAL VARIATION	17		$\frac{192.50}{9}$ = 21.39	

SPECIES: Praunus flexuosus ADULT

SITE: SALTS HOLE O_2 CONC. = 8 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		6.70	0.12
S_Q	1		22.6.65	39.76 **
SALINITY	2	2213.35		
T_L	1		4109.95	74.06 **
T_Q	1		804.17	14.49 **
TEMPERATURE	2	4914.12		
S_LT_L	1		1.39	0.03
S_LT_Q	1		9.98	0.18
S_QT_L	1		228.54	4.12 *
S_QT_Q	1		108.05	1.95
INTERACTION	4	347.96		
ERROR	9	7475.43	7974.80 - 7475.43 = 499.37	
TOTAL VARIATION	17	$\frac{499.37}{9}$ = 55.48		

SPECIES: Praunus flexuosus ADULT

SITE: HOLKHAM BAY O_2 CONC. = 2 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		758.27	40.29 **
S_Q	1		1566.44	83.23 **
SALINITY	2	2324.71		
T_L	1		807.54	42.90 **
T_Q	1		1267.24	67.33 **
TEMPERATURE	2	2074.78		
S_LT_L	1		631.19	33.53 **
S_LT_Q	1		20.90	1.11
S_QT_L	1		11.07	0.58
S_QT_Q	1		243.91	12.96 **
INTERACTION	4	907.07		
ERROR	9	5306.56	5475.91 - 5306.56 = 169.35	
TOTAL VARIATION	17		$\frac{169.35}{9}$ = 18.82	

SPECIES: Praunus flexuosus ADULT

SITE: HOLKHAM BAY O_2 CONC. = 5 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		493.69	23.48 **
S_Q	1		855.47	40.67 **
SALINITY	2	1349.16		
T_L	1		4147.06	197.28 **
T_Q	1		1334.28	63.46 **
TEMPERATURE	2	5481.74		
S_LT_L	1		38.68	1.84
S_LT_Q	1		5.09	0.24
S_QT_L	1		17.39	0.83
S_QT_Q	1		226.24	10.76 **
INTERACTION	4	287.40		
ERROR	9	7118.30	$7307.47 - \underline{7118.30} = 189.17$	
TOTAL VARIATION	17		$\frac{189.17}{9} = 21.02$	

SPECIES: Praunus flexuosus ADULT

SITE: HOLKHAM BAY O_2 CONC. = 8 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		483.23	17.56 **
S_Q	1		2247.55	81.69 **
SALINITY	2	2730.78		
T_L	1		4979.65	180.94 **
T_Q	1		994.04	36.12 **
TEMPERATURE	2	5973.50		
S_LT_L	1		48.95	1.78
S_LT_Q	1		0.04	0.00
S_QT_L	1		29.19	1.06
S_QT_Q	1		126.27	4.58 *
INTERACTION	4	204.45		
ERROR	9	8908.73	9156.39 - 8908.73 = 247.66	
TOTAL VARIATION	17		$\frac{247.66}{9}$ = 27.52	

SPECIES: Praunus flexuosus JUVENILE

SITE: SALTS HOLE O_2 CONC. = 2 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		73.56	1.36
S_Q	1		3846.27	71.35 **
SALINITY	2	3919.83		
T_L	1		1648.77	30.59 **
T_Q	1		3092.84	57.38 **
TEMPERATURE	2	4741.63		
S_LT_L	1		222.61	4.13 *
S_LT_Q	1		188.05	3.48 *
S_QT_L	1		997.43	18.51 **
S_QT_Q	1		95.45	1.77
INTERACTION	4	1503.54		
ERROR	9	10165.00	10650.12 - 10165.00 = 485.12	
TOTAL VARIATION	17		$\frac{485.12}{9}$ = 53.90	

SPECIES: Praunus flexuosus JUVENILE

SITE: HOLKHAM BAY O_2 CONC. = 2 mg/l.

Source of Variation	dF	Sum of Squares.	Mean Square	F. ratio
S_L	1		1393.42	26.60 **
S_Q	1		1657.98	31.63 **
SALINITY	2	3051.40		
T_L	1		643.87	12.29 **
T_Q	1		1211.27	23.12 **
TEMPERATURE	2	1855.14		
S_LT_L	1		130.82	2.50
S_LT_Q	1		128.11	2.45
S_QT_L	1		77.22	1.47
S_QT_Q	1		237.44	4.53 *
INTERACTION	4	573.58		
ERROR	9	5480.13	5951.51 - 5480.13 = 471.38	
TOTAL VARIATION	17		$\frac{471.38}{9}$ = 52.38	

APPENDIX NINE

ELECTROPHORESIS OF Lap and Mdh

ISOENZYMES

OF

Idotea chelipes

Gammarus duebeni

Fraunus flexuosus

PREPARING ENZYME EXTRACTS

1. Prepare buffer. This is Trizma-Barbital buffer.(Sigma No. 710-1). Each vial contains TRIZMA 0.05 mol.,Sodium barbital 0.05 mol., Barbital 0.014 mol..Each vial is dissolved in 750 ml of deionized water. The pH of the buffer should be 8.9 0.1 at 25°C.

2. Submerge 'Cellogel' plate in 250 ml of buffer for 15 minutes.

3. Place 750 ml of buffer in the tank,ensuring that the levels in each section are similar.

4. Eliminate excess buffer from the plate between two sheets of filter-paper and place the plate on the bridge of the tank. Connect the bridge supports and adjust the plate until it is firm.

5. Using the 1.5 µl sample-applicator,place samples of extract at the cathodic end.Repeat applications three times.

6. Run at 400 volts for 1 hour using a 'Vokam' or similar standard power supply. The current should be between 10 to 15 m amps.

7. On completion of the run,the plate is stained using one of the following methods.

LEUCINE AMINOPEPTIDASE.

The stain is made up in Tris maleate buffer at pH 6.0.This is produced by mixing 50 ml of solution A with 26 ml of solution B. Solution A is Tris 24.2 g ; maleic acid 23.2g ; H_2O to one litre. Solution B is 0.2M NaOH.

After mixing the appropriate quantities of A and B,the solution is made up to 200ml with deionized water.

The stain consists of:

Tris-maleate buffer pH 6.0	50ml.
Water	50ml.
Black K salt	50mg.
L-Leucyl -β- naphthylamide	20mg.

Incubate the plate in the stain at 35°C until dark bands appear.This is approximately 30mins.Wash and fix.

MALATE DEHYDROGENASE

The stain consists of:

NAD+	50mg.
NBT	330mg.
PMS	2mg.
1M Na L-malate :	100ml. *
0.5M Tris-HCl	15ml.
Water	70ml.
0.1M NaCN	5ml.

Incubate for one hour at 35°C.Wash and fix.

* (L-malic acid 13.4g ; 2M $Na_2CO_3.H_2O$ (248 g/l) 49ml,Water to 1 litre.

Method of calculating Genetic Identity (I), between two populations.

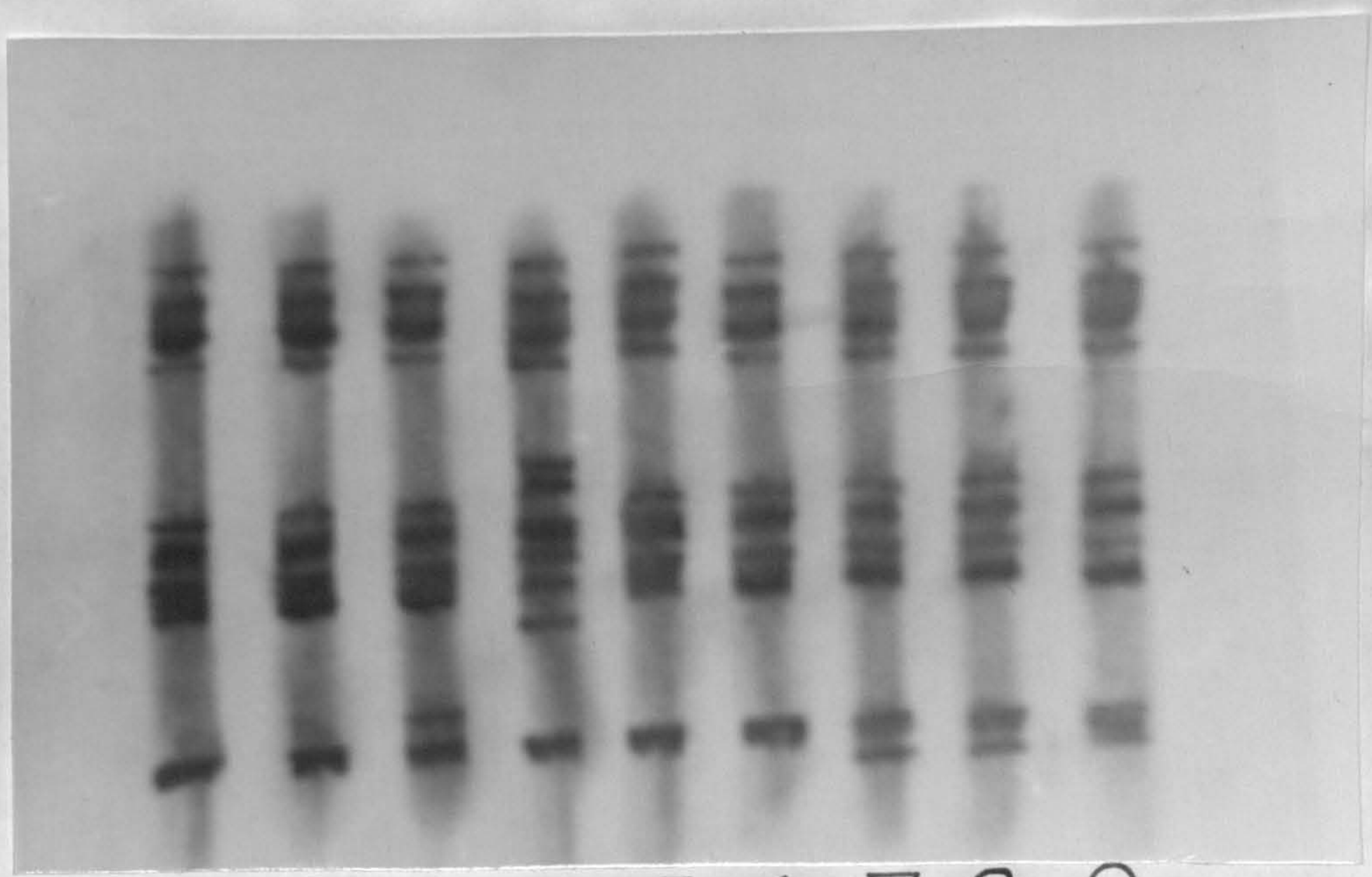
The example below is for populations of Idotea chelipes at stations 1 and 2.

ENZYME	LOCATION sta 1	LOCATION sta 2	$\Sigma x_i y_i$ (a)	Σx_i^2	Σy_i^2	$(\Sigma x_i^2 \Sigma y_i^2)$ (b)	$I_j = (a/b)$
Lap 1	1.00	1.00	1.0000	1.0000	1.0000	1.0000	1.0000
Lap 2	0.87	1.00					
	0.13	0.00	0.8700	0.7738	1.0000	0.8797	0.9889
Mdh 1	0.03	0.02					
	0.97	0.82					
	0.00	0.07					
	0.00	0.00	0.8833	0.9418	0.8334	0.8859	0.9971
Mdh 2	0.00	0.06					
	0.68	0.82					
	0.00	0.10					
	0.32	0.02	0.5640	0.5648	0.6864	0.6226	0.9059
Mdh 3	0.00	0.12					
	0.46	0.28					
	0.04	0.01					
	0.50	0.59					
	0.00	0.00					
	0.00	0.00	0.4242	0.4632	0.4410	0.4520	0.9385
Mean values:			0.7483	0.7487	0.7922	0.7702	0.9717

$\underline{I = 0.9717}$

A1. Expression of Mdh isoenzyme patterns in Idotea chelipes at station 4 - the Salts Hole.

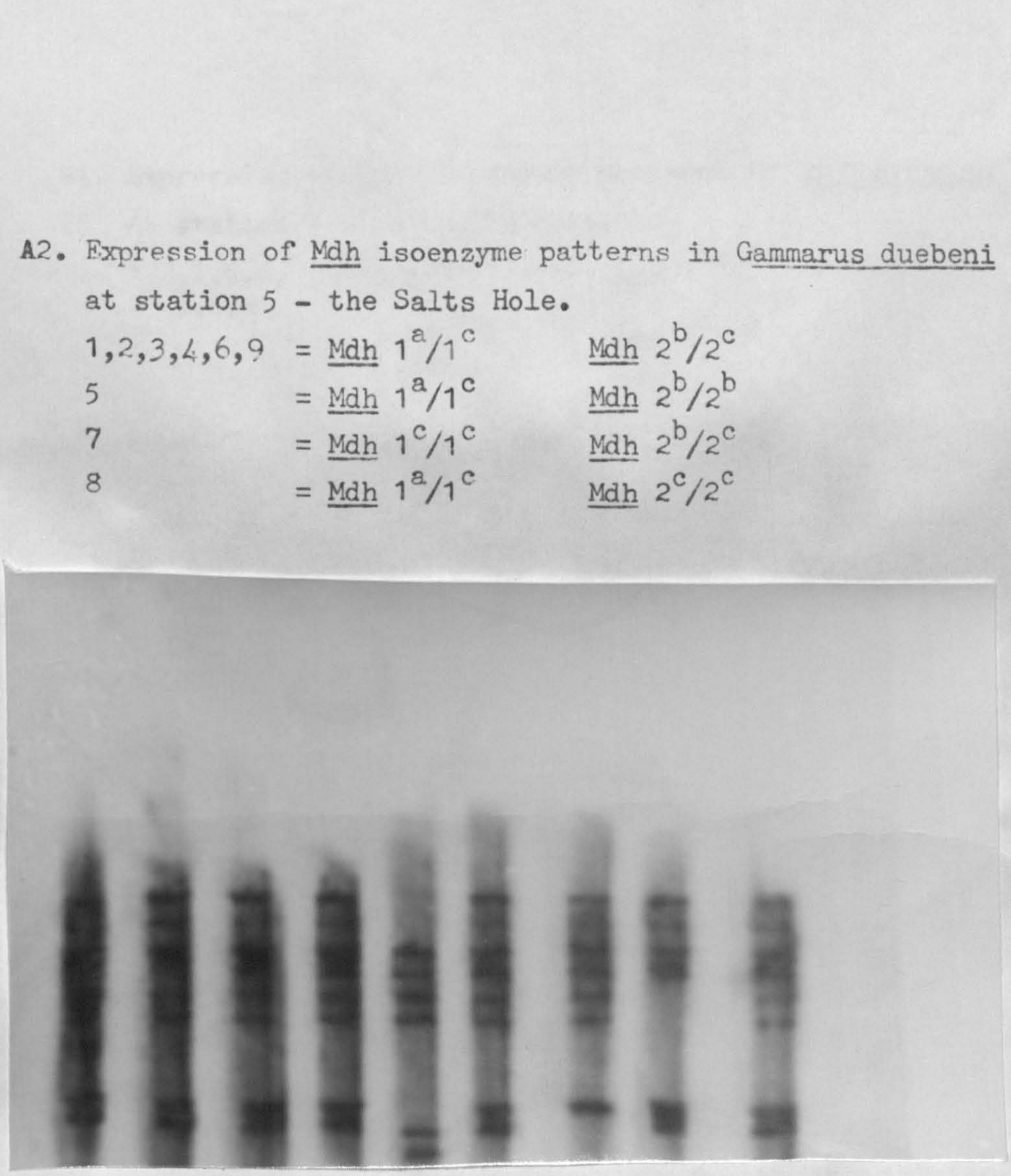
1,2,5,6	= Mdh $1^c/1^c$	Mdh $2^c/2^c$	Mdh $3^e/3^e$
3	= Mdh $1^c/1^d$	Mdh $2^c/2^c$	Mdh $3^e/3^e$
4	= Mdh $1^c/1^c$	Mdh $2^b/2^c$	Mdh $3^e/3^e$
7,8,9	= Mdh $1^b/1^c$	Mdh $2^c/2^c$	Mdh $3^e/3^e$



1 2 3 4 5 6 7 8 9

A2. Expression of Mdh isoenzyme patterns in Gammarus duebeni at station 5 - the Salts Hole.

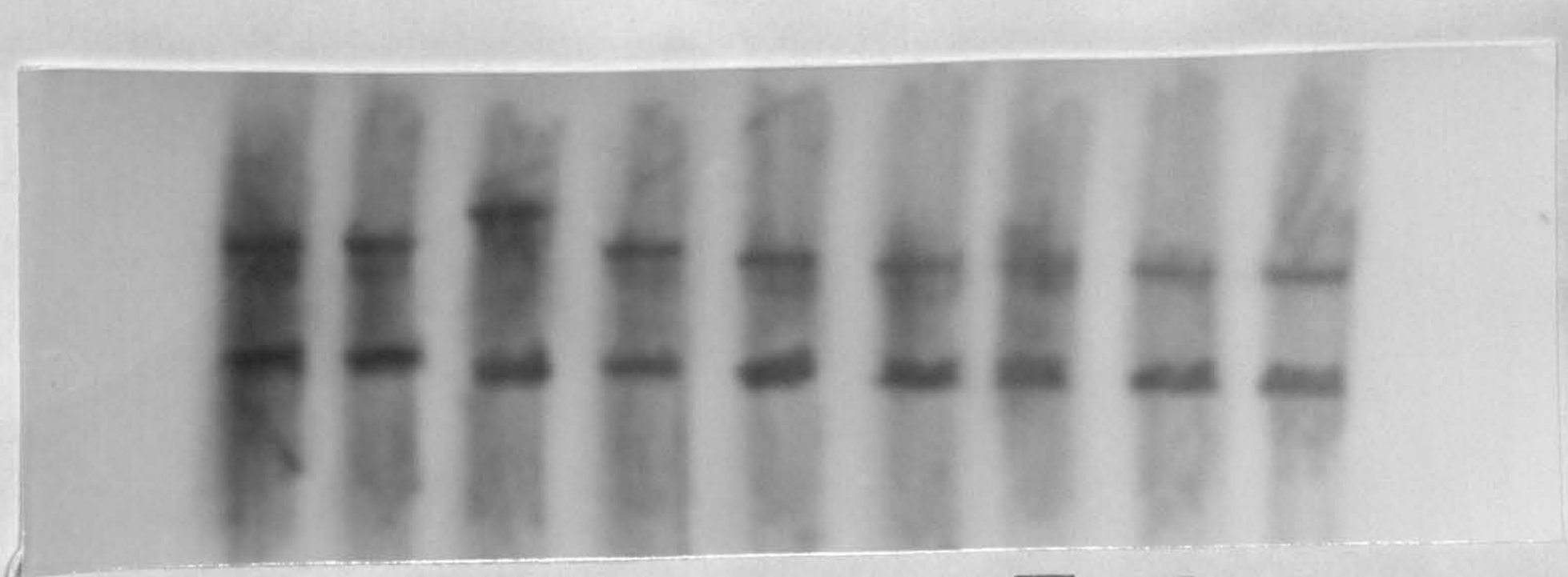
1,2,3,4,6,9	= Mdh $1^a/1^c$	Mdh $2^b/2^c$
5	= Mdh $1^a/1^c$	Mdh $2^b/2^b$
7	= Mdh $1^c/1^c$	Mdh $2^b/2^c$
8	= Mdh $1^a/1^c$	Mdh $2^c/2^c$



1 2 3 4 5 6 7 8 9

B1. Expression of Lap isoenzyme patterns in Idotea chelipes at station 5 - The Salts Hole.

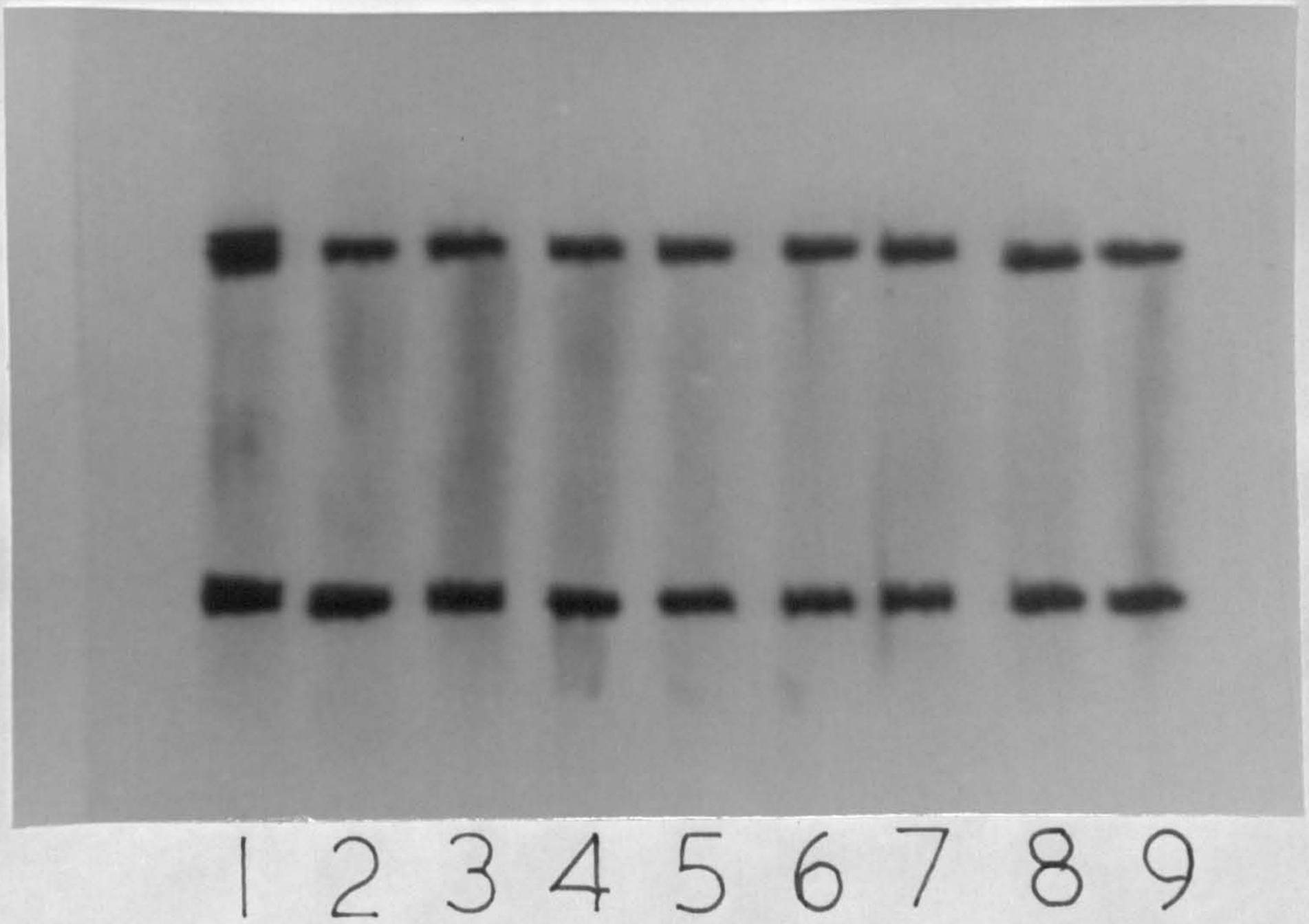
1,2,4,5,6,7,8,9	=	Lap $1^b/1^b$	Lap $2^b/2^b$
3	=	Lap $1^b/1^b$	Lap $2^a/2^a$



1 2 3 4 5 6 7 8 9

B2. Expression of Lap isoenzyme patterns in Praunus flexuosus, at station 4 - the Salts Hole.

1	=	Lap $1^b/1^c$	Lap $2^b/2^b$
2 - 9	=	Lap $1^b/1^b$	Lap $2^b/2^b$



1 2 3 4 5 6 7 8 9